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## Behavioral Responses of the Stem Borer *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae), First Instar Larvae to Some Environmental Factors

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**Abstract:** The behavior of first instar larvae of *C. partellus*, under some environmental conditions was studied. The caterpillars exhibited a strong photonegative response to the light. These larvae showed bimodal distribution, one to the north and the other one to the south, for the colored experimental design. The *C. partellus* first instar larvae, significantly moved faster during high light intensities

In the presence of their host plants they moved in all directions when the arena was surrounded by sorghum plants.

**Keywords:** Stem borer *Chilo partellus*, behavioural responses, environmental factors

### INTRODUCTION

The stem borer *Chilo partellus* (Swinhoe) is a major pest of sorghum and maize in many tropical countries including Africa. Better understanding of the interplay between insect movement and weather is particularly relevant to pest management.

A review of the effect of weather and weather elements on the dispersal of airborne organisms, insects has been published by Pedgley (1982).

Among all the factors that influence agricultural production, climate and soils are the most important. This is dramatic in Africa, where the high temperature and high humidity of equatorial Africa provide ideal conditions for pests and diseases (Treitz and Narain, 1988). Some works indicated that dispersal is not accidental but rather a distinct behavior through which organisms interact with spatial variation in their environment (Kennedy, 1951; Kennedy, 1956; Southwood, 1962; Johnson, 1969). Two main components of the environment: temperature and relative humidity influence insect behavior as exemplified in the aggregation of *Dysdercus* (Madge, 1965; Youdeowei, 1966; Youdeowei, 1967; Youdeowei, 1968) and *Dermestes maculatus*

Deg. (Toye, 1970). Glick (1942) stated that when there are great numbers of insects in the air, it is quite evident that certain meteorological conditions, particularly temperature, humidity and light, are definitely favorable to their occurrence.

There is a need for entomologists to understand the interaction between weather elements and insects pests behavior, in order to develop a system for forecasting the conditions conducive to crop pests mass transport, to determine the periods and locations of treatments and traps set up in the field.

Amano (1985) studying the influence of meteorological factors on the flight activity of Tabanids, indicated that in general, solar radiation, relative humidity and wind velocity affected flight activity of female Tabanids. The importance of individual factors varied by species. According to Andrewartha and Birch (1954), weather was recognized as influencing most invertebrates' behavior, development and survival, acting either directly or indirectly through the food supply. Some works pointed out that the temperature more than any other individual physical factor influences aerial dispersal of both winged and wingless invertebrates.

There is a temperature threshold below which insects are unable to take of Taylor (1963) and a lower one below which they are unable to fly (Wellington, 1954; Cockbain, 1961). However there is no published work on the effects of environmental factors on the orientational behavior of *Chilo partellus* first instar larvae.

The aims of the present investigation were to study *Chilo partellus* first instar larvae responses to weather elements in their immediate environment.

## MATERIALS AND METHODS

The experiments were carried out at ICIPE, Mbita Point Field Station, in the uncultivated plots at different locations. First instar larvae of *C. partellus* used, were collected from the stock laboratory culture maintained on an artificial diet (Ochieng *et al.*, 1985). A portable weather station, which consisted of an anemograph to indicate and record wind-speed and wind direction, a photometric measuring unit for a direct read of light intensity, a wet and dry bulb hygrometer which records temperature and relative humidity range, a compass to determine the exact sun position.

Larval behavioral orientation was studied using a hard paper board (120 × 120 cm). To the surface of the paper was drawn with a pencil, two circles (20 and 40 cm diameter respectively), having the same center. These circles were divided into twelve sections of 30° each (Fig. 1).

A batch of 10 first instar larvae was released at the start point. Ten minutes later, the number of larvae out of each circle, their orientation and displacement (by reading off the sections in which they were found) were recorded every 15 minutes for 2 hours. Apart from wind whom direction is easterly mornings and westerly evenings every day, were also recorded sun position, light intensity. After each run a fresh batch of 10 first instar insects was used for a following trial. The paper on which the experiment was conducted, was changed after each replicate. This was repeated four times. Observations on larvae that did not cross the first circle were not included in the analysis.

Three different colored paper boards (white, green and pink) were used to test the effect of reflectance on larval movement. In the second set of these studies, potted sorghum plants either surrounded the experimental arena or were placed north and

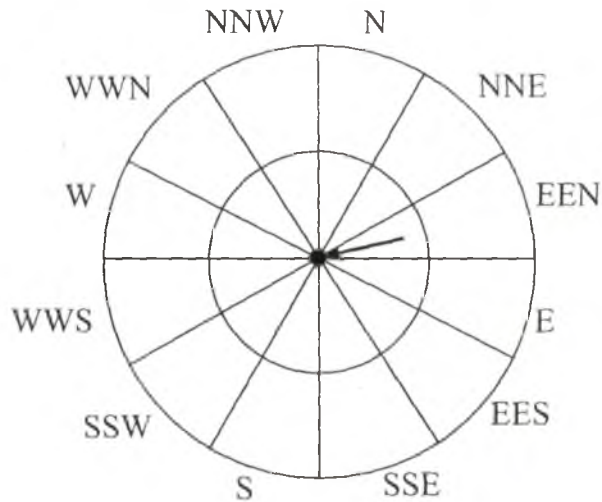


Fig. 1. Experimental design for behavioral study of first instar larvae of *C. partellus*. (—): start point where the larvae were released before each test. Letters represent different directions N: north, NNE: north/northeast, EEN: east/east-north, E: east, EES: east/east-south, SSE: south/southeast, S: south, SSW: south/southwest, WWS: west/west-south, W: west, WNW: west/west-north, NNW: north/northwest.

south (60 cm apart). The all experiment design surrounded by an opaque brown mat enclosure (4 cm high, 8 cm wide), 2 m from the edge of the experimental design in order to avoid any visual stimuli effects.

All experiments were conducted during mornings (08:00–10:00 a.m.) and evenings (04:00–06:00 p.m.). Data was subjected to analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was used for comparison of means (Sas Institute, 1982).

## RESULTS

During the period of the experiments, the sun position was east/east-south (EES) mornings and west/west-south (WWS) evenings. Whereas the wind direction recorded before each test was easterly and westerly respectively.

A bimodal distribution, of the *Chilo partellus* first instar larvae, was obtained for each colored experimental arena used, one to the north direction and the other to the south direction. Statistical analysis with DMRT ( $P < 0.05$ ) showed no significant difference from on another (Fig. 2a, 2b, 2c). Therefore any reflectance effect was not shown on the larval movement.

According to the sun position mentioned above, the orientation of the first instar larvae mornings and evenings was  $150^\circ$  and  $90^\circ$  respectively to the source of light indicating a strong negative phototaxis during this larval stage.

Observations, during the highest light intensities, of the behavior of the larvae showed that they moved faster than those in cooler weather, that suggested an orthokinesis. The light intensities recorded varying from 42,000 to 75,000 lux mornings and from 10,000 to 65,000 lux evenings (Fig. 3). Dixon and Mercer (1983) observed that the proportion of Aphids takeoff increases with light intensity up to 1,000 lux. Conversely, the light and temperature threshold for takeoff for second generation of adults

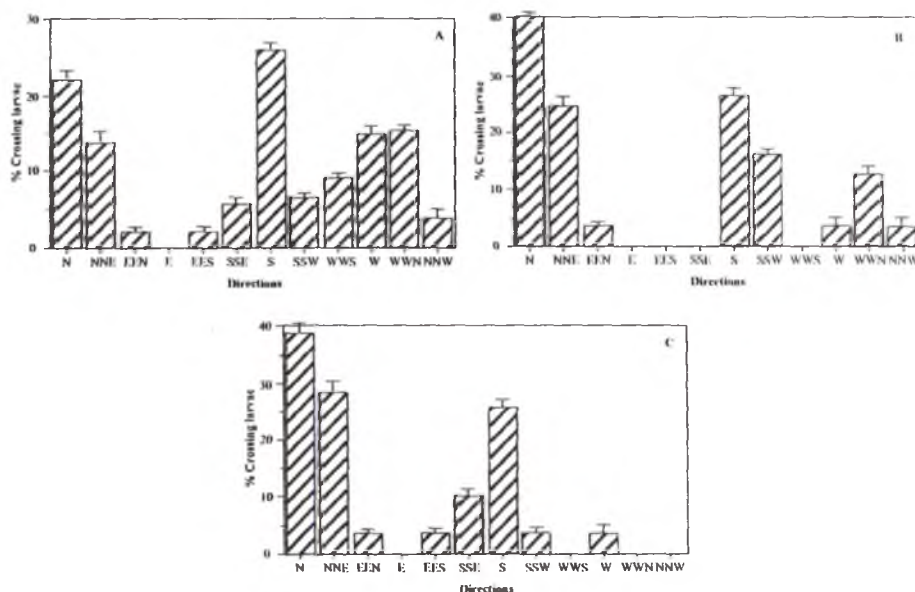


Fig. 2A, 2B, 2C. Distribution of *C. partellus* first instar larvae in a free choice situation, on a white (A), green (B) and pink (C) experimental arena. Vertical bars represent standard errors.

are lower, enabling them to fly in the morning, and (Duelli, 1984) found that adults of the lacewing (*Chrysoperea carnea*) hide in vegetation until illumination falls below about 20 lux.

Larvae moved a distance of 16 and 25 cm (away from light) in 20 and 40 seconds respectively as compared to 3 and 5 cm in cooler weather.

In the presence of their host plants, *C. partellus* first instar larvae, become “thermotropic”, moving in all directions when the arena was surrounded by sorghum plants (Fig. 4a), regardless sun position and light intensities, whereas their distributions were bimodal, one peak to the north and the second one to the south (Fig. 4b) when host plants were in those directions.

The experimental area was surrounded with an opaque brown mat enclosure to eliminate any visual and olfactory stimulation. In such situation, larvae were distributed almost equally in the main directions (N, E, S, W) (Fig. 5).

There is evidence that, some of the informations obtained from this work, would be useful to explain aspects of *C. partellus* larvae behavior in the field and to determine the proper environmental conditions for a convenient experimental design.

## DISCUSSION

Southwood (1962) recognized two distinct types of insects movements: migratory and trivial. Migratory movements take an insect from the habitat from which it emerged and result in an increase in the mean distance between individuals from the source population. In migratory movement insect will not respond to vegetative stimuli (mate, food, shelter).



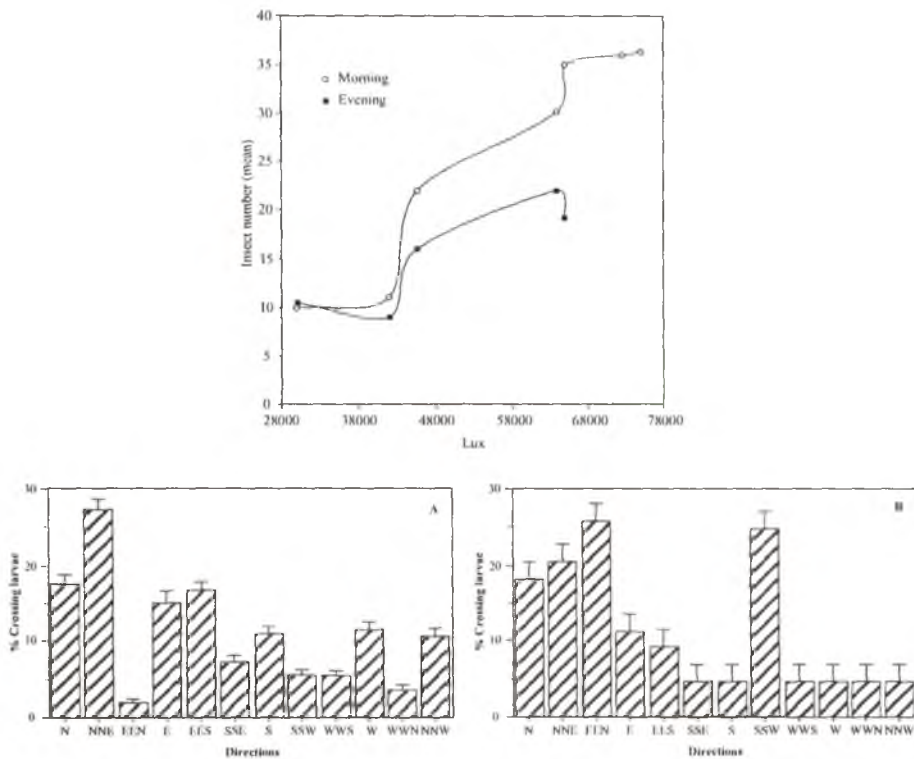


Fig. 3. The reactions of first instar larvae of *C. partellus* to light intensity gradient.

Fig. 4A, 4B. Distribution of first instar larvae of *C. partellus* in the presence of sorghum plants surrounding the experimental arena (A) or only in north and south directions (B). Vertical bars represent standard errors and letters as for Fig. 2.

Trivial movements are usually restricted to the insect's original habitat, it is variable and may be terminated at any time upon an insect's perception of a vegetative stimulus. The same author used the term "Passive movement" to refer to airborne individuals that have no control over the direction or duration of their flight. In general and as it has been shown in the present work, *C. partellus* larvae exhibited a trivial movement, due to visual and olfactory stimulations (Tokro and Saxena 1991).

As well as plant and insect variables, the environments of the assay arena itself can affect the validity of experimental results and the effects of lighting, temperature and humidity should be carefully determined (Smith, 1978). According (Barnes and Ratcliffe, 1967), light has been shown to affect test results to the extent that strong photopositive or photonegative response of the insect can override both olfactory and gustative stimuli.

Our investigations indicated that *C. partellus* first instar larvae shown a photonegative response to light. Greene and Morril (1970), found also a strong photonegative response in the behavioral orientation of the larvae of the cabbage loopers *Trichoplusia ni* (Hübner). Taylor (1965) started that within the boundary layer, insects may move downwind unless they perceive some other strong directional element such as food,

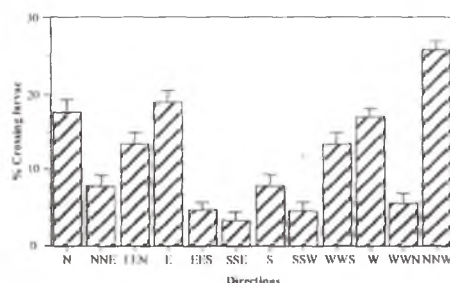


Fig. 5. Distribution of first larvae of *C. partellus* on an experimental arena, in the middle of an opaque brown mat enclosure. Vertical bars represent standard errors and letters as for Fig. 2.

oviposition sites or pheromone.

Boiteau (1986) stated that the periodicity of takeoff and flight varied among three species of aphids on potatoes, significant peaks of activity occurred in the morning and early afternoon for the green peach aphid, *Myzus persicae* (Sulzer), in the morning for the potato aphid, *Macrosiphum euphorbiae* (Thomas) and in the afternoon for the buck thorn aphid *Aphis nasturtii*.

Dispersal is not random event but rather a dynamic product of insect behaviour and mesoscale weather systems, and the concentration of pest insects by convergent wind systems and their eventual deposition can have a major impact on population dynamics and the incidence of economic damage. An important goal of Integrated Pest Management Programs in both agriculture and forestry is to forecast changes in population over times so that actions can be instituted to prevent or ameliorate economic damage (McManus, 1988).

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## Fecundity and Diurnal Oviposition Behaviour of Sorghum Shoot Fly, *Atherigona soccata* Rondani (Diptera: Muscidae)

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**Abstract:** The fecundity and diurnal oviposition behaviour of sorghum shoot fly, *Atherigona soccata* Rondani was studied on CSH 5, a susceptible sorghum genotype under greenhouse conditions. Fecundity of shoot flies deprived of 10 day-old sorghum seedlings was drastically reduced, and no fertile eggs were laid after 11 days of host deprivation. However, when provided with host plants, egg production was prolonged, and dropped considerably after the flies were 22 day-old. There were three distinct peaks (6 & 7, 13 & 14, and 18 & 19 days of age) in egg laying activity during adult life span. Further studies with flies of three age groups (7, 13, and 19 day-old) did not show significant differences in diurnal oviposition pattern with age. However, most eggs (60%) were laid between 0800 and 1200 hours. It was evident from the present study that the prolonged egg laying vitality of shoot fly adult when provided with a susceptible host may partly account for the dramatic increase in shoot fly damage as the crop season advances, since there would be a geometric increase in active females with every new generation of flies.

**Keywords:** Shoot fly, *Atherigona soccata*, sorghum genotypes, fecundity, rate of increase, deadheart, oviposition behaviour.

### INTRODUCTION

Sorghum, *Sorghum bicolor* (L.) Moench is an important cereal crop in Africa and Asia. Grain yields on farmers' fields are generally low (500-800 kg ha<sup>-1</sup>), insect pests being one of the major factors limiting sorghum production. Nearly 150 insect species have been reported as pests of sorghum (Seshu Reddy and Davies, 1979; Jotwani *et al.*, 1980). Sorghum shoot fly, *Atherigona soccata* Rondani (Diptera: Muscidae) is one of the most destructive pest of grain sorghum, which attacks 7 to 28 day-old sorghum seedlings (Nwanze *et al.*, 1990). The females lay white, elongated, cigar-shaped eggs

singly on the undersurface of the leaves, parallel to the midrib. The eggs hatch in 1-2 days, and the larvae crawl along the leaf lamina to reach the plant whorl and then move downward through the central shoot till they reach the growing point. They cut the growing point and feed on the decaying leaf tissues, resulting in deadheart formation. The female has a life span of 30 days.

Oviposition is a biological response which to a large extent is influenced by the genotype of the host plant (Sharma *et al.*, 1990). In order to evaluate sorghum genotypes for resistance to the shoot fly, it is necessary to obtain information on fecundity on a susceptible host under greenhouse conditions (Raina, 1982). The present investigations were undertaken to study the influence of withholding oviposition by shoot fly on fecundity, successful infestation, larval survival, and adult emergence, when deprived of and provided with host plant for a better understanding of the rate of increase of the pest during the cropping season. The present paper includes shoot fly diurnal oviposition behaviour in relation to female longevity.

## MATERIALS AND METHODS

### Influence of withholding Oviposition by Shoot Fly on Fecundity, Successful Infestation, Larval Survival, and Adult Emergence

Susceptible CSH 5 plants were grown in twelve plastic baby bath tubs ( $42 \times 30 \times 14$  cm). Each tub accommodated 50 plants with a spacing of  $15 \times 5$  cm between rows, and plant hills. When the seedlings were 10 day-old, the tubs were placed in the first compartment ( $82 \times 75 \times 60$  cm) of a 3-compartment cage and were artificially infested with field collected gravid shoot fly females. The adults were confined with the plants for 12 h. Ten days after infestation, all the deadhearts were harvested and kept in moist sand in a metal tray ( $60 \times 30 \times 10$  cm) placed in the central compartment ( $65 \times 75 \times 60$  cm) of the cage for adult emergence. Newly emerged adults entered through a funnel into the last compartment ( $52 \times 75 \times 60$  cm) and were fed on diet (brewer's yeast powder glucose in 1 : 1 ratio and cotton soaked in 10% sucrose solution in a petri-dish). To collect the flies from the last compartment, it was covered with a thick black cloth (close weave) leaving the collection container as the only source of light. Adult flies collected were kept in a cage ( $40 \times 35 \times 30$  cm) and provided with diet.

After the pre-oviposition period of three days, 10 females were collected every day from the cage, and released on 10 day-old seedlings for 12 h. The females were deprived of sorghum seedlings at 1 day interval for a duration of 18 days under greenhouse conditions at  $26-30 \pm 3^\circ\text{C}$ ,  $60-75 \pm 5\%$  RH, and a photoperiod of 12 : 12 (L:D) hours. Observations were recorded on retention duration (days), total number of plants, plants with eggs, total number of eggs, number of plants with unhatched eggs, number of deadhearts, number of dead larvae, number of dead pupae, and number of emerged adults. The biological and damage parameters were calculated as follows:

$$\begin{aligned} \text{Fecundity} &= \frac{\text{total number of eggs}}{\text{number of females released}} \\ \% \text{ successful infestation} &= \frac{\text{total number of deadhearts}}{\text{total plants with eggs}} \times 100 \end{aligned}$$

% larval survival

$$= \frac{\text{total no. of deadhearts} - \text{total no. of dead larvae}}{\text{total no. of deadhearts}} \times 100$$

Proportion of adult emergence from deadheart plants

$$= \frac{\text{total number of emerged adults}}{\text{total number of deadhearts}} \times 100$$

### Pattern of Shoot Fly Oviposition in Relation to Female Longevity

To obtain shoot fly culture, same procedure as above was adopted. After the pre-oviposition period of three days, 25 gravid females were collected from the cage, and released (5 females/2 pots/cage) on 10 day-old seedlings, provided with diet and were allowed to lay eggs for 24 h. Twenty-four hours after exposure, the seedlings were replaced daily with fresh ones, till death of all adult flies. Observations were recorded on the number of eggs laid per female per day.

### Temporal and Diurnal Patterns of Shoot Fly Ovipositional Behaviour in Relation to Female Age

To obtain shoot fly culture, same procedure as above was adopted. This experiment was conducted based on the results obtained from pattern of shoot fly oviposition in relation to female longevity under greenhouse conditions. Shoot fly females in three distinct age groups (7, 13, and 19 day-old) were used.

After the pre-oviposition period of three days, 15 gravid females of each age group were collected from the rearing cage and released (5 females/2 pots/cage) on 10 day-old seedlings at 2 h intervals for about 24 h. Twenty-four hours after exposure, the seedlings were replaced daily with fresh ones, till death of all adult flies. The experiment was replicated three times and observations were recorded on the number of eggs laid every 2 h. Data were statistically analysed using a Least Significant Difference (LSD) test at 5% probability level.

## RESULTS

### Influence of withholding Oviposition by Shoot Fly on Fecundity, Successful Infestation, Larval Survival, Adult Emergence

Fecundity of shoot flies deprived of sorghum seedlings was drastically reduced, and no fertile eggs were laid after 11 days of host deprivation, even when subsequently provided with host plants (Table 1). Percent successful infestation *i.e.*, expression of deadhearts dropped considerably after 11 days of host deprivation. A great percentage of the larvae survived from 1-11 days. Same trend occurred in adult emergence.

### Pattern of Shoot Fly Oviposition in Relation to Female Longevity

However, when provided with host plants, egg viability and successful development were prolonged, and dropped considerably after the flies were 22 day-old (Table 2). More than 90% of eggs hatched. There were three distinct peaks (based on weekly intervals) in egg laying activity during the adult life span. Peaks in oviposition occurred at 6 & 7, 13 & 14, and 18 & 19 days of age, and were as high as 4.5 eggs female<sup>-1</sup> day<sup>-1</sup>.

Table 1: Effect of Withholding Oviposition by Host Deprivation by Shoot Fly on Biological Parameters

Adult age (days)	Retention duration (days)	Fecundity Eggs/female	% successful infestation	% larval survival	Proportion of adult emergency
1	Pre-ovi	—	—	—	—
2	"	—	—	—	—
3	"	—	—	—	—
4	1	5.2	76.7	69.0	29.7
5	2	4.3	85.8	89.7	32.8
6	3	4.4	81.8	87.8	14.8
7	4	2.6	67.8	72.0	26.0
8	5	3.6	48.8	58.0	32.0
9	6	4.7	89.8	79.0	36.0
10	7	1.9	72.7	25.0	40.0
11	8	1.3	40.0	20.0	20.0
12	9	2.4	67.8	54.8	54.7
13	10	1.9	24.0	22.2	26.0
14	11	2.8	52.8	21.7	30.0
15	12	1.2	0.0	0.0	0.0
16	13	3.9	2.2	0.0	0.0
17	14	4.0	2.3	0.0	0.0
18	15	2.1	0.0	0.0	0.0
19	16	1.7	0.0	0.0	0.0
20	17	1.3	0.0	0.0	0.0
21	18	0.6	0.0	0.0	0.0

### Temporal and Diurnal Patterns of Shoot Fly Ovipositional Behaviour in Relation to Female Age

Further studies with flies of three age groups (7, 13, and 19 days) did not show significant differences in diurnal oviposition pattern with age. However, most eggs (60%) were laid between 0800 and 1200 hours (Table 3).

### DISCUSSION

The number of eggs laid per shoot fly female when provided with host plant has been examined in several studies (Kundu and Kishore, 1970; Raina, 1982), but the subsequent impact on eggs resulting from host deprivation was not investigated in these studies. Therefore, experiments on the fecundity of shoot fly deprived of and with access to sorghum seedlings provided useful information in determining the rate of increase of the pest. There was evidence of a decrease in fecundity of shoot fly when deprived of sorghum seedlings, and after 11 days of host deprivation, the eggs laid were infertile. Whereas flies with access to host plants had longer duration in eggs laying activity during adult life span and more than 90% of the eggs hatched. Sorghum shoot fly is host specific (Ogwaro, 1978; Raina and Kibuka, 1983) and gravid females used in the present study did not prefer to lay eggs on cage walls or in petri-dishes containing diet in the cage, when deprived of sorghum. The fecundity of 62 eggs/female in a period of 1-19 days obtained in the present study when shoot flies had access to seedlings was lower than the 78 eggs/female reported by Raina (1982) on CSH 1. Meksongsee *et*



Table 2: Pattern of Shoot Fly Oviposition in Relation to Female Longevity (days)

Adult age (days)	Oviposition duration (days)	No. of females		Total No. of eggs laid	Average no. of eggs per female		% eggs hatched
		Alive	Laid eggs		1/*	2/**	
1	Pre-ovi	—	—	—	—	—	—
2	"	—	—	—	—	—	—
3	"	—	—	—	—	—	—
4	1	25	25	181	7.2	7.3	99.2
5	2	25	15	34	1.4	2.3	100.0
6	3	25	25	151	6.0	6.0	98.8
7	4	25	20	124	5.0	6.2	96.8
8	5	25	15	107	4.3	7.2	99.8
9	6	25	15	20	2.0	3.4	92.0
10	7	25	25	140	5.6	5.6	97.8
11	8	25	25	62	2.5	2.5	95.2
12	9	20	14	40	2.0	2.9	100.0
13	10	20	14	65	3.3	4.6	93.8
14	11	20	18	91	4.6	5.1	92.7
15	12	16	8	12	0.8	1.5	100.0
16	13	8	0	0	0.0	0.0	0.0
17	14	8	3	4	0.5	1.3	100.0
18	15	7	6	36	5.1	6.0	72.7
19	16	7	7	27	3.9	3.9	88.8
20	17	7	7	13	1.9	1.9	100.0
21	18	5	5	7	1.4	1.4	100.0
22	19	1	1	5	5.0	5.0	100.0

\* Number of eggs laid/female based on the number of females alive

\*\* Number of eggs laid/female based on actual number of females that laid eggs

Table 3: Temporal and Diurnal Patterns of Shoot fly Ovipositional Behaviour in Relation to Female Age

Time interval (hours)	Number of eggs laid		
	Age of adult females (days)		
	7 day-old	13 day-old	19 day-old
0600 - 0800	2.0bc	1.0cd	0.0d
0800 - 1000	15.7a	8.3a	5.3a
1000 - 1200	14.7a	6.7ab	3.8b
1200 - 1400	11.7ab	5.3b	2.7bc
1400 - 1600	7.3abc	2.7c	2.0c

Means within a column followed by the same letter are not significantly different at  $P=0.05$  using a Least Significance Difference (LSD) test.

*al.* (1978) reported from Thailand that the shoot flies laid an average of 235 eggs. The variations in fecundity could be due to differences in exposure periods, time interval to locate a host plant, adult longevity due to different environmental conditions, and diet fed to the adults.

Studies on diurnal oviposition pattern of flies show that up to 60% of the total eggs were laid between 0800 and 1200 hours. This was associated with photoactive

stimuli (e.g. colour) and optimum temperature and relative humidity. It was evident from the present study that the prolonged egg laying vitality of shoot fly adult when provided with a susceptible host may partly account for the dramatic increase in shoot fly damage as the crop season advances, since there would be a geometric increase in active females with every new generation of flies.

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## Differential Uptake of Storage Proteins by the Fat Body of Rice moth, *Corcyra cephalonica*, During the Larval–Pupal Development

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**Abstract:** Three storage proteins (SPs) have been identified in *Corcyra cephalonica* with molecular weights of 86 kDa (SP 1), 84 kDa (SP 2) and 82 kDa (SP 3). Fat body of last instar larvae show a low uptake of all the three SPs. During the prepupal stage of development, there is a significant increase in SPs uptake by the fat body and SP 1 is sequestered preferentially. However, during the early-pupal development the SPs uptake remains generally high but SP 2 and SP 3 are sequestered distinctly more than SP 1. Sodium azide, sodium fluoride and low temperature treatments inhibit SPs uptake. Juvenile hormone I also inhibits SPs sequestration by the fat body cells *in vitro*, while 20-hydroxyecdysone stimulates the uptake of SPs. The present study clearly suggests a stage dependent selective uptake of SPs during post-embryonic development of *Corcyra cephalonica*, which is regulated by ecdysteroid.

**Keywords:** Storage proteins, fat body, uptake, *Corcyra cephalonica*

### INTRODUCTION

In holometabolous insects, the fat body synthesizes large number of proteins including storage proteins (SPs) and release them into haemolymph during active feeding phase of last larval stadium (Eussudies and Law, 1983; Levenbook, 1985; Kanost, *et al.*, 1990; Telfer and Kunkel, 1991; Tojo and Yoshiga, 1993). These proteins are variously designated as hexamerins (Telfer and Kunkel, 1991), larval haemolymph proteins (Ray, *et al.*, 1987) or larval serum proteins (Roberts, *et al.*, 1977). The protein synthesizing function of the fat body greatly diminishes after cessation of feeding (Martin, *et al.*, 1971; Sekeris, and Scheller, 1977; Pau, *et al.*, 1979; Tojo, *et al.*, 1982; Powell, *et al.*, 1984; Caglayan and Gilbert, 1987; Ray, *et al.*, 1987) and it starts sequestering SPs (Eussudies and Law, 1983; Marinotti and deBianchi, 1986) which are deposited in the form of protein granules (Tojo, *et al.*, 1978; Ueno and Natori, 1982). The process of SPs uptake is mediated by receptors present in the plasma membrane of the fat body cells (Ueno, *et al.*, 1983). Several studies in diptera (Ueno and Natori, 1984;

Matzner and Scheller, 1989; Burmester and Scheller, 1992; Burmester and Scheller, 1995; Chung, *et al.*, 1995) and lepidoptera (Webb and Riddiford, 1988a; Webb and Riddiford, 1988b) showed that ecdysteroids stimulate SPS uptake by the fat body.

In *Corcyra cephalonica*, larval fat body synthesizes three SPS with molecular weight of 86 kDa (SP 1), 84 kDa (SP 2) and 82 kDa (SP 3). Juvenile hormone I (JH I) inhibits synthesis of SP 1 and SP 2, while 20-hydroxyecdysone (20E) stimulates SPS synthesis in *Corcyra* (Ismail and Dutta-Gupta, 1988; Ismail, 1991). Recently we have demonstrated the presence of specific binding proteins in the fat body membranes of *Corcyra*, which mediate the sequestration of SP during the late-larval and pupal stage of development (Kiran Kumar, *et al.*, 1997). Further we have also demonstrated the sequestration and secretion of intact SPS by the male accessory reproductive glands of *Chilo partellus* which is stimulated by 20E and its non-steroidal ecdysone agonist RH 5849 (Ismail and Dutta-Gupta, 1991; Ismail, *et al.*, 1993). In this paper we present evidence for a differential uptake of SPS by fat body cells during the last larval, prepupal and pupal development and also report on the effect of certain metabolic inhibitors, temperature and hormones on the *in vitro* SPS uptake by the fat body cells.

## MATERIALS AND METHODS

### Insects

*Corcyra cephalonica* larvae were mass reared in the laboratory at  $26 \pm 1^\circ\text{C}$  temperature,  $70 \pm 5\%$  relative humidity, 14 : 10 h light dark period on coarsely crushed sorghum seeds. For the present study, mid-last and late-last instar larvae, prepupae and early-pupae were used. The larval stages were identified by head capsule size and body weight as stated in our earlier publications (Ashok and Dutta-Gupta, 1988; Lakshmi and Dutta-Gupta, 1990).

### Biolabelling of storage proteins

Mid-last instar larvae were injected with  $10 \mu\text{Ci}$  [ $^{35}\text{S}$ ] methionine (specific activity-8000 Ci/m mol, Amersham) and incubated for 16 h. Haemolymph was collected in an eppendorf tube prerinsed with 0.1% phenylthiourea. It was then centrifuged at  $10,000 \times$  for 2 min at  $4^\circ\text{C}$  to remove the haemocytes. The supernatant was used for SDS-PAGE analysis which was carried out according to the procedure of Laemmli (1970) using 7.5% gel. SPS were identified and excised separately from the gels and electroeluted using 10 mM phosphate buffer (pH 7.4). The electroeluted SPS were dialyzed against 50 mM Tris-glycine buffer (pH 8.3) and concentrated by lyophilization. The specific activity of each SP was determined using scintillation spectrophotometry. Protein was estimated by method of Lowry *et al.*, (1951). The homogeneity of electroeluted SPS was checked by SDS-PAGE.

### Purification of SP

It was purified from haemolymph of mid or late-last instar larvae. The haemolymph was diluted (1 : 20) with insect Ringer (130 mM NaCl, 5 mM KCl, 0.1 mM  $\text{CaCl}_2$ ) containing 0.01% phenylthiourea and centrifuged to remove the haemocytes. The supernatant was added with ammonium sulfate to make 40% saturation and was centrifuged at  $10,000 \times$  g. The supernatant obtained was raised to 60% ammonium sulfate saturation and was centrifuged at  $10,000 \times$  g. The supernatant was raised again to 70%

ammonium sulfate saturation and centrifuged at  $10,000 \times g$ . The pellet thus obtained was suspended in insect Ringer and passed through Sephadex G-25 column for desalting. Peak fractions were pooled and subjected to DE-52 column. The proteins were eluted with a linear gradient of 0 to 1 M NaCl. Peak fractions were pooled and dialyzed against insect Ringer. The purity of the sample was checked on SDS-PAGE and stored at  $-20^{\circ}\text{C}$  after lyophilization.

### **Biotin labelling of SP**

Protein was biotinylated using Boehringer Mannheim Biotin labelling kit (Germany).

### ***In vivo* uptake studies**

[ $^{35}\text{S}$ ] Methionine labelled SP preparations were injected into the haemolymph of late-last instar, prepupae and early-pupae. The insects were sacrificed 12 h after injection. The fat body was dissected out, rinsed thoroughly in insect Ringer and was processed for determination radioactivity using Beckman liquid scintillation counter.

### ***In vitro* fat body culture and low temperature treatment**

For *in vitro* studies the fat body was dissected out from the prepupae and incubated in 100  $\mu\text{l}$  Grace's medium (Sigma Chem. Co., USA) at  $25^{\circ}\text{C}$  in sterile condition. Known amounts of biolabelled SPS were added to the incubation medium and cultures were incubated for 8 h. For low temperature treatment, incubation was carried out at  $4^{\circ}\text{C}$ . For certain *in vitro* studies, biotin labelled SPS were added to fat body cultures for 8 h. The fat body was rinsed thoroughly and processed for SDS-PAGE and subsequently proteins were transferred on to a nitrocellulose membrane (Towbin, *et al.*, 1979). Biotinylated SPS were visualized with a streptavidinperoxidase complex using chemiluminescence detection.

### **Inhibitor and hormone treatment**

Metabolic inhibitors, such as sodium azide and sodium fluoride were selected for the present study. Sodium azide (0.5  $\mu\text{g}$  in 5  $\mu\text{l}$  distilled water) or of sodium fluoride (5  $\mu\text{g}$  in 5  $\mu\text{l}$  distilled water) was added to the *in vitro* cultures of fat body. The fat body was collected after 8 h treatment and the recovered radioactivity was determined. For hormone treatment, either 0.25  $\mu\text{g}$  of 20E (in 2  $\mu\text{l}$  of 10% ethanol) or 1  $\mu\text{g}$  of JH I (in 1  $\mu\text{l}$  acetone) was added to the fat body cultures. The tissue was collected 8 h after incubation and processed for the measurement of radioactivity.

## **RESULTS**

### **Changes in *in vivo* uptake of SPS by the fat body during the postembryonic development**

The uptake of [ $^{35}\text{S}$ ] methionine labelled SPS by the fat body during last larval, prepupal and early-pupal development is presented in Figure 1. There was no significant uptake of radiolabelled SPS by the fat body during last larval development. However, the fat body of the prepupae and early-pupae showed a significant uptake of SPS. The amount of SP 1 sequestered by prepupal fat body was 3 to 4 fold higher as compared to SP 2 and SP 3. On the other hand, the fat body of early-pupa incorporated less of SP 1 than the prepupal fat body and showed a preferential uptake of SP 2 and SP 3.

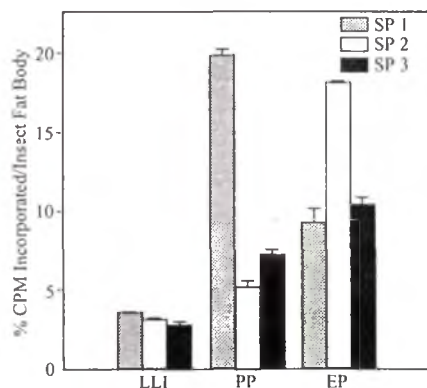


Fig. 1.

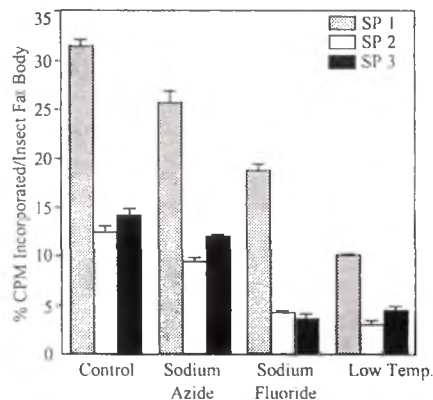


Fig. 2.

Fig. 1. *In vivo* uptake of [ $^{35}$ S] methionine labelled SPs by the fat body of *Corcyra cephalonica* during the late-last larval (LLI), prepupal (PP) and early-pupal (EP) development.

Insects were injected with biosynthetically labelled SPs and sacrificed 12 h after injection. The specific activity of SP 1 was 6,921 CPM/ $\mu$ g protein, SP 2 was 6,578 CPM/ $\mu$ g protein and SP 3 was 6,784 CPM/ $\mu$ g protein. Amount of SP injected/insect was 7.0  $\mu$ g - SP 1, 8.0  $\mu$ g - SP 2 and 6.4  $\mu$ g - SP 3. The fat body was dissected out and processed for radiolabel measurement. The values represent the  $\pm$  S.D. of three different experiments. For each experiment two insects were used.

Fig. 2. Effect of sodium azide, sodium fluoride and low temperature on *in vitro* uptake of SPs by prepupal fat body of *Corcyra cephalonica*.

The fat body was dissected out from the prepupae and cultured *in vitro* in 100  $\mu$ l Grace's medium at 25°C in sterile condition with biosynthetically labelled SPs. The specific activity of SP 1 was 6,921 CPM/ $\mu$ g protein, SP 2 was 6,578 CPM/ $\mu$ g protein and SP 3 was 6,784 CPM/ $\mu$ g SP 3. The fat body was treated either with 0.5  $\mu$ g sodium azide or with 5  $\mu$ g sodium fluoride for 8 h. For low temperature treatment fat body cultures were incubated at 4°C for 8 h. The values represent the  $\pm$  S.D. of three different experiments.

### Effect of inhibitors on the *in vitro* uptake of SPs

Figure 2 shows the effect of metabolic inhibitors: sodium azide and sodium fluoride on the *in vitro* uptake of SP 1, SP 2 and SP 3 by the prepupal fat body. The data shows that 0.5  $\mu$ g sodium azide treatment for 8 h resulted in lowering the values of the measured SP uptake to 16%, 22% and 19% respectively. The fat body treated with 5  $\mu$ g sodium fluoride also showed reduced uptake of SPs. Unlike sodium azide, the inhibition of uptake of SP 2 and SP 3 was more drastic than SP 1 in sodium fluoride treated fat body cultures.

### Effect of temperature on the *in vitro* uptake SPs

In order to determine the effect of temperature on SPs sequestration, the fat body from prepupae was incubated in Grace's medium at 4°C for 8 h. The results presented in Figure 2 show that exposure to low temperature reduced the uptake of all the three categories of SPs by the fat body cells and the inhibition ranged between 68–77%.

### Effect of hormones on the *in vitro* uptake SPs

The fat body from prepupae was incubated with biolabelled SPs the presence of either 0.25  $\mu$ g of 20E or 1  $\mu$ g JH 1 and the results are set forth in Figure 3. JH 1 treatment for 8 h partially blocked the uptake of all the three SPs by the prepupal fat body and



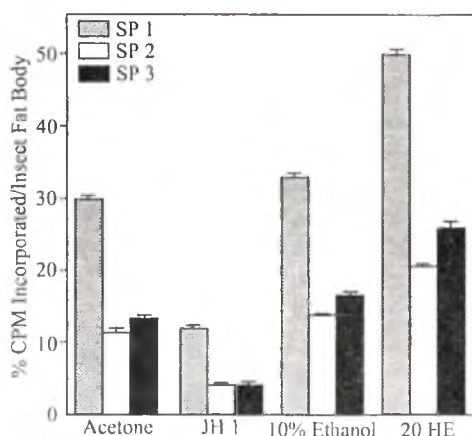


Fig. 3.



Fig. 4.

Fig. 3. Effect of JH I and 20E on *in vitro* uptake of SPs by prepupal fat body of *Corcyra cephalonica*.

The fat body was dissected out from the prepupae and cultured *in vitro* in 100  $\mu$ l Grace's medium at 25°C in sterile condition with biosynthetically labelled SPs. The fat body was either treated with 1  $\mu$ g of juvenile hormone I (JH I) in 1  $\mu$ l acetone or with 0.25  $\mu$ g 20-hydroxyecdysone (20E) in 1  $\mu$ l of 10% ethanol for 8 h. The specific activity of SP 1 was 6,921 CPM/ $\mu$ g protein, SP 2 was 6,578 CPM/ $\mu$ g protein and SP 3 was 6,784 CPM/ $\mu$ g protein. Amount of SP added/insect fat body was 7.0  $\mu$ g - SP 1, 8.0  $\mu$ g - SP 2 and 6.4  $\mu$ g - SP 3. These values represent the  $\pm$  S.D. of three different experiments.

Fig. 4. Western blot pattern of biotinylated storage proteins obtained after *in vitro* uptake by the prepupal fat body of *Corcyra cephalonica*. Lane 1 - control in presence of biotinylated SPs, lane 2 - experimental in presence of biotinylated SPs + 0.25  $\mu$  20E (in 2  $\mu$ l of 10% ethanol) and lane 3 - control (-biotinylated SPs). Note the presence of intact SPs ( $\rightarrow$ )

the uptake was reduced by 60–65% following JH I treatment. On the other hand, 20E treatment for 8 h, significantly stimulated the uptake of all the three SPs by the fat body. However, the stimulatory effect was more pronounced in the case of SP 3.

Figure 4 illustrates the SDS-PAGE pattern of biotinylated SPs obtained from the prepupal fat body after *in vitro* uptake studies. This figure clearly shows that SPs are sequestered in intact form and retain their molecular weights (lane 1), and the uptake is significantly stimulated in the presence of 20E (lane 2).

## DISCUSSION

The fat body from late-last instar larvae of *Corcyra cephalonica* was able to incorporate only a small amount of biolabelled SPs injected into haemolymph. The selective uptake of SPs into the fat body cells at the end of the larval development after cessation of feeding, is well documented in lepidopteran insects (Miller and Silhacek, 1982; Tojo, *et al.*, 1982; Caglayan and Gilbert, 1987). Earlier studies from our laboratory revealed that the fat body from the last instar larvae of *Corcyra cephalonica* was able to incorporate only small amounts of radiolabelled SP under *in vitro* as well as *in vivo* conditions and this process could be stimulated by 20E (Ismail and Dutta-Gupta, 1990; Kiran Kumar, *et al.*, 1997). We have also demonstrated the sequestration and secretion of intact SPs by the male accessory reproductive glands of *Chilo partellus* which could be stimulated by 20E as well as its non-steroidal agonist RH 5849 (Bajaj,

*et al.*, 1990; Ismail and Dutta-Gupta, 1991; Ismail, *et al.*, 1993). Present *in vitro* uptake studies using biotinylated SPS, provide a direct evidence that all the three SPS are sequestered in intact form.

Our data presented here shows that the fat body of prepupae and early-pupae actively incorporate the injected biolabelled SPS from the haemolymph. The uptake of SPS by the fat body is a selective process in the present insect, because at the prepupal stage SP 1 is incorporated to a greater extent than SP 2 and SP 3. Interestingly there is a reversal of this trend as the development advances to early-pupal stage. Undoubtedly, remarkable amounts of all the three SPS continue to be sequestered also by the early-pupal fat body but with difference that the amount of SP 1 incorporated at this stage is much lower than that in the prepupal stage and at the same time, the early-pupal fat body sequestered more SP 2 and SP 3 in comparison to SP 1. In *Galleria mellonella*, 74 and 76 kDa storage proteins are sequestered during the spinning stage but not the 81 and 82 kDa proteins (Miller and Silhacek, 1982). However, both the storage proteins P 1 and P 2 are taken up by the prepupal fat body of *Manduca sexta*, with a five fold higher uptake of P 1 (Caglayan and Gilbert, 1987). The present *in vivo* uptake experiments on *Corcyra cephalonica* provide a direct evidence for the sequestering ability of fat body and accumulation of SPS during the prepupal and early-pupal stage, which is developmentally regulated. In diptera and lepidoptera the process of uptake is mediated by binding proteins and/or specific receptors present in the plasma membranes of fat body cells which are activated by ecdysteroids (Ueno and Natori, 1984; Chung, *et al.*, 1995; Burmester and Scheller, 1992; Wang and Haunerland, 1993; Wang, and Haunerland, 1994).

Marinotti and deBianchi (1987) showed that SPS uptake by the fat body *Musca domestica* requires active metabolism and can be partially blocked by metabolic inhibitors. *In vitro* uptake of all the three SPS by the fat body of *Corcyra cephalonica* is partially inhibited by sodium azide and sodium fluoride. The uptake of SPS is lowered further when the fat body cultures are incubated at low temperature at 4°C instead of 25°C. This fact suggests that the binding of SP to its binding protein and/or receptor in the fat body cell is adversely affected by low temperature.

Our present study shows that JH I inhibits the *in vitro* uptake of SPS by the prepupal fat body. This is in accord with the study on *Bombyx mori* where it was shown that the JH analogue, methoprene inhibited the fat body storage protein uptake (Tojo, *et al.*, 1982). On the contrary, it is seen here that incubation of *Corcyra cephalonica* prepupal fat body with 20E significantly stimulates the uptake of all the three SPS and once again the amount of SP 1 incorporated is more than that of SP 2 and SP 3. These results suggest that 20E not only mediates the sequestration of SPS during post-embryonic development but also regulates the differential uptake of SPS in a stage specific manner. Using native SP and ligand blotting technique, recently we have identified two SP protein binding proteins with apparent molecular weight of 125 and 120 kDa in the larval fat body membrane of *Corcyra cephalonica* (Kiran Kumar, *et al.*, 1997). Further studies are necessary to clarify how these binding proteins/receptors are activated for SPS uptake, whether or not the same binding protein recognizes the three different SPS. It would be then interesting to look in to the intrinsic structural features of these SPS which confer specificity for their uptake at different developmental stage.

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## Quantitative and Qualitative Performance of Nistari and Bivoltine Hybrid Combinations of Silkworm *Bombyx mori* L.

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**Abstract:** A comparative study of yield performance of Nistari hybrids evolved by Nistari and 4 popular bivoltines (NB<sub>4</sub>D<sub>2</sub>2, NB<sub>18</sub>, KA and CC<sub>1</sub>) has been carried out both in favourable (Sep–Oct) and unfavourable (May–June) seasons. The results indicated that, the performance of different crosses were on par or superior to PM × NB<sub>4</sub>D<sub>2</sub> and on par or inferior to N × NB<sub>4</sub>D<sub>2</sub>. Cumulative variable index (CVI) indicated that hybrids N × (NB<sub>4</sub>D<sub>2</sub> × NB<sub>18</sub>) followed by N × (NB<sub>4</sub>D<sub>2</sub> × KA), N × NB<sub>4</sub>D<sub>2</sub>, N × (NB<sub>18</sub> × KA) and N × NB<sub>18</sub> × NB<sub>4</sub>D<sub>2</sub> emerged as the best five combinations in the order of merit. Overall performance of rearing and reeling characters showed that hybrids involving Nistari as a female component was superior to PM × NB<sub>4</sub>D<sub>2</sub>.

**Keywords:** Silkworm, Rearing and reeling parameters, hybrid

### INTRODUCTION

In the eastern and North-Eastern regions of India, indigenous multivoltine race Nistari is still dominating the silk industry. It is a hardy race with shorter larval duration, yielding reliable crops, but with low productivity and poor silk quality (Subba Rao, 1988; Goldsmith, 1991). However, the silk production is being improved by introducing the hybrids of Nistari × Pure bivoltines. For various reasons, the production of bivoltine pure races may not be adequate, compelling the seed producers to use bivoltine hybrids as male parent. On the other hand, bivoltine hybrids exhibited better tolerance, increased vigour, easy to rear by the farmers with higher silk content and shorter larval duration when compared to pure bivoltines (Subba Rao, 1988; Das *et al.*, 1994). The hybrids of Nistari × (bivoltine × bivoltine) have been proposed as suitable combinations during warmer seasons – June–July and August–September in West Bengal (Subba Rao, 1988). Keeping this in view, Nistari being a hardy multivoltine race and a good combiner, a study on the possibility of exploiting bivoltine hybrid as male parent was carried out. The results are compared with PM × NB<sub>4</sub>D<sub>2</sub> and Nistari × NB<sub>4</sub>D<sub>2</sub>.







Table 2: Cumulative variable index for ranking N X Bivoltine hybrid combinations

Sl. No.	Combinations	RANKING		
		Favourable season	Unfavourable season	Combined for two seasons
1.	N × KA	15	2	8
2.	N × (KA × CC <sub>1</sub> )	16	10	11
3.	N × (KA × NB4D2)	12	9	10
4.	N × (KA × NB18)	7	3	6
5.	N × CC <sub>1</sub>	11	4	7
6.	N × (CC <sub>1</sub> × KA)	14	6	9
7.	N × (CC <sub>1</sub> × NB18)	13	15	16
8.	N × (CC <sub>1</sub> × NB4D2)	10	14	14
9.	N × NB18	9	13	13
10.	N × (NB18 × NB4D2)	4	7	5
11.	N × (NB18 × KA)	5	5	4
12.	N × (NB18 × CC <sub>1</sub> )	8	12	12
13.	N × NB4D2	3	8	3
14.	N × (NB4D2 × NB18)	2	1	1
15.	N × (NB4D2 × KA)	1	11	2
16.	N × (NB4D2 × CC <sub>1</sub> )	6	16	15
17.	PM × NB4D2	17	17	17

## MATERIALS AND METHODS

A polivoltine Nistari, four bivoltine races NB4D2, NB18, KA and CC<sub>1</sub> and all possible hybrids of these bivoltines were utilised for this study. The female parent Nistari was crossed with bivoltine pure and their hybrids, as shown in the Table 2. These hybrids were reared in two seasons, *i.e.*, favourable (Sep–Oct) and unfavourable (May–June) seasons in three replications of 500 worms each, maintained after 3rd moult. Performance data on 12 important commercial characters *viz.*, yield/10,000 larvae by No., (ERR by no.) Yield/10,000 larvae by Wt., (ERR by wt), single cocoon wt., single shell wt., shell ratio, absolute silk content, reelability, filament length, filament wt., denier, non-breakable filament length (NBFL) and silk percentage (on dry cocoon wt.,) were recorded. The results are compared using standard statistical procedure with PM × NB4D2 and N × NB4D2 combinations. CVI is generated by working out a selection index method suggested by Hector *et al.*, 1991. In this method, the variables are weighed equally with positively contributing variables are given positive weightage and negatively contributing variables are given negative weightages. The smaller the index, better will be the performance of the combination.

## RESULTS

Mean performance of the hybrids combined for two seasons is presented in Table 2. None of the combinations were significantly different from PM × NB4D2 in respect of ERR by no., ERR by wt., cocoon wt., and denier. However, the performance of combinations in respect of shell wt., shell ratio, absolute silk content, reelability, filament length, filament wt., and NBFL were on par or superior to PM × NB4D2. Excepting in two combinations (N × KA and N × CC<sub>1</sub>) the percentage of silk also

exhibited the similar trend.

When the results are compared with  $N \times NB4D2$ , the characters like ERR by no., ERR by wt., and denier did not vary in all the combinations. However, in respect of cocoon wt., shell wt., shell ratio, absolute silk content, reelability, filament length, filament wt., [except  $N \times (NB4D2 \times NB18)$ ] and NBFL were on par or inferior to  $N \times NB4D2$ . Significantly higher and lower silk % was noticed in 4 crosses each and the remaining were on par with  $N \times NB4D2$ . It could be seen from Table 1, cumulative variable index worked out for all the parameters studied indicated that hybrids  $N \times (NB4D2 \times NB18)$  followed by  $N \times (NB4D2 \times KA)$ ,  $N \times NB4D2$ ,  $N \times (NB18 \times KA)$  and  $N \times (NB18 \times NB4D2)$  emerged as the best five combinations. The performance of two crosses viz.,  $N \times (NB4D2 \times NB18)$  and  $N \times (NB18 \times KA)$  were found to be better in both the seasons. Among all the combinations, the performance of  $PM \times NB4D2$  ranked as the poorest combinations in both favourable and unfavourable seasons.

During favourable season the performance of all the hybrids in respect of ERR by no., and ERR by wt. were on par with  $PM \times NB4D2$ . In respect of shell wt., shell ratio, and filament wt., all the sixteen combinations were significantly higher than  $PM \times NB4D2$ . Absolute silk content and filament length were significantly higher in ten and nine combinations respectively. When the results are compared with  $Nistari \times NB4D2$ , none of the combinations were significantly different in respect of ERR by no., ERR wt., cocoon wt., shell ratio, absolute silk content and denier. Cumulative variable index showed that hybrids,  $N \times (NB4D2 \times KA)$  followed by  $N \times (NB4D2 \times NB18)$ ,  $N \times NB4D2$ ,  $N \times (NB18 \times NB4D2)$  and  $N \times (NB18 \times KA)$  emerged as the best five combinations during this season (Table 1).

During unfavourable season, none of the combinations were significantly different from  $PM \times NB4D2$  in respect of ERR by no., ERR by wt., shell wt., absolute silk content and denier. However, the performance of all the crosses in respect of reeling parameters viz., reelability, filament length, filament wt., and NBFL were significantly superior to  $PM \times NB4D2$ . Comparison of results with  $N \times NB4D2$  indicated that the performance of different combinations in respect of ERR by no., ERR by wt., cocoon wt., shell ratio, reelability, filament length, filament wt., denier, NBFL and silk % were on par with  $N \times NB4D2$ . Cumulative variable index showed that hybrids  $N \times (NB4D2 \times NB18)$  followed by  $N \times KA$ ,  $N \times (KA \times NB18)$ ,  $N \times CCI$  and  $N \times (NB18 \times KA)$  emerged as the best five combinations for this season (Table 1).

## DISCUSSION

The results obtained from this investigation showed that, the mean performance of different crosses were on par or superior over  $PM \times NB4D2$  and on par or inferior to  $N \times NB4D2$  in most of the traits studied. This experiment demonstrated the superiority of Nistari hybrids over  $PM \times NB4D2$  in respect of rearing and reeling parameters. This may be attributed to the fact that Nistari may be a good combiner with bivoltines than Pure Mysore.

The performance of different characters during two distinct seasons demonstrated the superiority of Nistari three way crosses over  $PM \times NB4D2$ . However, during favourable season the performance of different crosses were on par or inferior to  $N \times NB4D2$  which indicates the superiority of  $N \times NB4D2$  during this season. But,

during unfavourable season most of the characters like ERR By no., ERR By wt., Cocoon wt., Shell ratio, reelability, filament length, filament weight, denier, NBFL and silk % in different hybrid combinations studies were on par with N × NB4D2. These results corroborated with the findings of Subba Rao (1988) who proposed three way crosses of Nistari as suitable hybrids for warmer seasons in West Bengal. Moreover, it is well accepted that, rearing of bivoltine hybrid as foundational seed is more easier than pure strain under high temperature conditions and the males of F<sub>1</sub> hybrids could be conveniently used in the production of three way cross. These advantages are clearly demonstrated by Singh and Hirobe (1964). Sato (1975) opined that, three way crosses are well suited to warmer seasons due to their inherent ability to utilise rich protein of mulberry leaf. The overall results indicated that, Nistari × Bivoltine hybrid combinations have better tolerance, more vigour with negligible segregation and their performance were on par with ruling hybrid N × NB4D2. Hence, these hybrids could be exploited during warmer season to utilise the available mulberry leaf for higher cocoon production.

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## Immunological Identification of Vitellogenin and Regulatory Mechanisms Involved in Ovarian Development of *Achaea janata* (Lepidoptera: Noctuidae)

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**Abstract:** Immunodiffusion studies were conducted to identify vitellogenin (Vg) in *Achaea janata*. Vitellogenin synthesis began in 4 day old 5th instar larva, while uptake of vitellogenin by developing oocytes was noticed in 5 day old pupa which continued till 8th day. Rudimentary ovarioles devoid of oocytes were noticed in thorax ligated individuals 5 days after ligation. Injection of 20-hydroxyecdysone (20-HE) has great influence in enhancing the growth of ovarioles and increasing the number of oocytes. This effect was dose dependant.

**Keywords:** Vitellogenin, 20-hydroxyecdysone, ligation, *Achaea janata*

### INTRODUCTION

In most Lepidoptera, vitellogenesis occur independently of feeding and egg production appears to be a programmed response to the imaginal molt. In some Lepidoptera, mature eggs were produced in spite of allatectomy in the pupae (Sahota, 1977). However, there are other lepidopteran species where JH is required for vitellogenesis, if the adults feed before egg maturation. Among Coleoptera, JH is necessary to set off the initial vitellogenic response, then continued yolk protein production become autonomous (Dortland, 1979). In *Apis mellifera*, queens show no dependence on JH or ecdysone for the production of vitellogenins; although in this species both workers and drones are presumably JH dependent (Ramamurthy and Engels, 1977; Trenczek and Engels, 1986). In *Drosophila*, 20-HE is the primary trigger of yolk protein synthesis in the fat body, while JH is responsible for regulating Vg synthesis by the follicle cells, as well as acting as a secondary factor in the fat body and as a regulator of yolk protein uptake by the oocyte (Postelthwait and Shirk, 1981; Wu, *et al.*, 1987). Inspite of the

great diversity encountered, a unified scheme of the interplay between corpora allata, fat body and the ovary is accomplished by autoinhibition and short and long-loop feed back (Belles, 1995).

Earlier studies have shown that vitellogenesis in *Achaea janata* occur during the pupal stage (Nair and Muraleedharan, 1992). In the present paper an attempt was made to elucidate the mechanism of Vg synthesis in the fat body, its uptake by the developing oocytes and also the hormonal regulatory mechanism involved in the physiology of egg maturation.

## MATERIALS AND METHODS

### Insects

*A. janata* were reared in the laboratory as described earlier (John and Muraleedharan, 1989).

### Immunodiffusion

Immunodiffusion techniques were performed to identify the time of vitellogenin (Vg) synthesis and their sequestration into the hemolymph and developing oocytes. The technique described by Johnstone and Thorpe (1987) is employed in the present investigations. Freshly laid eggs were washed in saline and homogenized. Yolk contents from 75 eggs were used at a time for immunizing a rabbit. The homogenate was centrifuged, and the supernatant was used as the antigen. Injections were made subcutaneously at the thigh region. Control group was injected with saline. Sera were obtained from both control and immunized rabbits and these constituted the control antiserum and anti-yolk serum respectively.

Extracts of hemolymph and fat body of both male and female and ovary from females were prepared in saline and were tested for the presence of Vg or vitellin (Vt) by allowing them to react with anti-yolk serum. Control serum is also subjected to immunodiffusion with the different tissue extracts parallel to test experiments.

### Ligation

0-hr old female pupae were ligated between the head and the prothorax (neck ligation) and between prothorax and mesothorax (thorax ligation) with fine hair loops and were kept in closed petri dishes. Non-ligated pupae were kept as control.

### Ligation and 20-Hydroxyecdysone Treatment

Thorax ligated insects were used to investigate the role of 20-Hydroxyecdysone (20-HE) on ovarian development and vitellogenesis. Two experimental groups viz. experimental group A and experimental group B were administered 5  $\mu$ g and 20  $\mu$ g of 20-HE respectively. The hormone was injected into the dorsal side of the abdomen of 0-day old ligated female pupae, after refrigeration for 5 to 10 minutes, by a microlitre syringe (Hamilton). Control groups were administered with appropriate quantities of insect saline.





Fig. 1. Immunoprecipitation of fat body proteins by anti-yolk serum a- fat body (Day 4, V instar); b-fat body (Day 1 pupa); A- anti-yolk serum.

Fig. 2. Immunoprecipitation of hemolymph proteins by anti-yolk serum c- hemolymph (Day 2 pupa); d- hemolymph (Day 3 pupa); e - hemolymph (Day 4 pupa).

Fig. 3. Immunoprecipitation of ovarian proteins by anti-yolk serum f- ovary (Day 6 pupa); g- ovary (Day 7 pupa); h- ovary (Day 8 pupa).

## 20HE Treatment to Normal Female Pupae

0-day old female pupae from the stock culture were taken and used to trace out the role of 20-HE on ovarian development and vitellogenesis. Two experimental groups viz. Experimental Group 1 and Experimental Group 2 were administered  $5\text{ }\mu\text{g}$  and  $20\text{ }\mu\text{g}$  of 20-HE respectively. Control groups were given with appropriate quantities of insect saline.

## RESULTS

### Immunological Studies

#### *Control Antiserum*

The tissue extracts of fat body and hemolymph from male and female along with ovaries at different maturation stages were added in the circumferential wells and the control antiserum in the central well. None of the above tissues reacted positively with the control antiserum.

#### *Anti-Yolk Serum*

The anti-yolk serum reacted positively with the female fat body of 4 day fifth instar larva (spinning stage) and 1 day old pupa resulting in the formation of precipitin line (Figure 1). But no reaction was observed with the male and female fat body of other instars and day 1, day 2 and day 3 fifth instar. Hemolymph of day 1, day 2, day 3 and day 4 pupae showed precipitin line with anti-yolk serum (Figure 2). The intensity of precipitation increased from day 2 pupa. Positive reaction was observed with the ovaries of day 5, day 6, day 7, day 8 pupa and anti yolk serum (Figure 3). A very poor reaction depicted by a very faint precipitin line between anti-yolk serum and the ovary of day 5 pupa was observed. Darkly stained precipitin lines were seen in the reaction between anti-yolk serum and ovary of day 8 pupa, ovary of adults and with freshly laid eggs.

Table 1: Effect of Ligation on the Development of Ovary in *A. janata* Pupa Ligated '0' Hour After Emergence (Mean  $\pm$  SD)

Position of ligation	Day after ligation	Status of ovary	
		Length of ovariole (mm)	No. of oocytes/ovariole
Neck	Day 5 @	20.50 $\pm$ 0.837* (20.0 $\pm$ 1.549)	27.50 $\pm$ 0.548* (28.167 $\pm$ 0.75)
	Day 8	44.50 $\pm$ 0.837* (43.67 $\pm$ 1.033)	162.33 $\pm$ 2.251* (160.0 $\pm$ 0 $\pm$ 2.0)
Prothorax	Day 5	R(19.66 $\pm$ 1.967)	0.0(29.50 $\pm$ 1.76)

@ Oocyte formation and differentiation of ovariole into germarium and vitellarium begin on day 5 pupa

Control values given in parentheses

R - Rudiments; \* not significant

Table 2: Effect of 20-HE on Mortality and Metamorphosis After Treatment on 1 Day Old Pupa

Dose of Hormone treated	No. of insects used	% Mortality	Further Development
5 $\mu$ g	40	10%	Survived pupae normally eclosed into adults
20 $\mu$ g	50	50%	Survived pupae not eclosed into adults
Control (Insect Saline)	50	0.00	Eclosed into adults

### Ligation and Ovarian Development

The length of ovariole and number of oocytes per ovariole in neck ligated insects had no significant difference from that of controls (Table 1). Rudimentary ovarioles without oocytes appeared in thorax-ligated insects 5 days after ligation. Prothorax ligation and subsequent 20-HE treated groups as well as their controls all died within one or two days after the treatment.

### 20-HE and Ovarian Development

5  $\mu$ g of 20-HE treated group recorded a mortality rate of 10% upto day 7 and the survived pupae emerged out as normal adults 9 days after treatment (Table 2). In 20  $\mu$ g of 20-HE treatments, upto 7 days of pupation, 50% mortality rate was recorded and the remaining pupae survived upto day 9.

Group 1 have not shown any significant difference with their controls in the length of ovariole, length of germarium and number of oocytes per ovariole (Table 3). In Group 2 the ovarioles appeared thread like upto fourth day and then a rapid growth and development of ovaries occurred from 6 day old pupa. Maximum elongation of ovariole was reported in 8 day old pupae. Oocyte formation has taken place from day 5 to day 8 of pupation in both treatments and in their corresponding controls. Group 2 showed high significance on the number of oocytes per ovariole from day 5 to day 8 with their controls (Table 3). In group 1 there was no significant difference in the length of germarium with their respective controls whereas Group 2 showed significance on day 5, day 6 and day 8 (Table 3). Group 2 noticed a gradual decrease in the length of germarium from 5 day through 8 day old pupa.

Table 3: Effect of 20-HE on the Length of Ovariole, Length of Germarium and Number of Oocytes/Ovariole (Mean  $\pm$  SD)

Characters	Group of insects	5th day pupa		6th day pupa	7th day pupa	8th day pupa
		5 $\mu$ g@	20 $\mu$ g			
Length of Ovariole (mm)	Experiment	19.40 $\pm$ 1.34 <sup>NS</sup>	24.00 $\pm$ 1.41**	40.60 $\pm$ 2.70*	49.40 $\pm$ 2.88*	68.40 $\pm$ 2.70*
	Control	20.40 $\pm$ 2.61	20.40 $\pm$ 2.61	34.40 $\pm$ 1.95	35.20 $\pm$ 1.48	49.20 $\pm$ 2.86
F value		0.581	7.364	17.315	96.019	118.916
Length of Germarium (mm)	Experiment	9.20 $\pm$ 0.84 <sup>NS</sup>	7.00 $\pm$ 1.00**	5.20 $\pm$ 0.84*	4.40 $\pm$ 0.55 <sup>NS</sup>	3.40 $\pm$ 0.55*
	Control	9.60 $\pm$ 1.67	9.60 $\pm$ 1.67	8.40 $\pm$ 1.67	4.60 $\pm$ 0.56	5.00 $\pm$ 0.00
F value		0.229	8.895	14.629	0.333	42.667
No. of oocytes $\pm$ ovariole	Experiment	32.60 $\pm$ 2.51 <sup>NS</sup>	96.80 $\pm$ 5.07*	141.20 $\pm$ 6.53*	217.00 $\pm$ 5.15*	263.00 $\pm$ 5.87*
	Control	34.20 $\pm$ 2.77	34.20 $\pm$ 2.77	83.80 $\pm$ 6.18	130.20 $\pm$ 3.70	163.40 $\pm$ 5.03
F value		0.914	586.641	203.632	937.095	829.445

@ 5 $\mu$ g 20-Hydroxyecdysone treated insects were studied only on day 5 pupa as it has no effect on ovarian development in *Achaea janata*

\*\* Significance at 5% level; NS - Not significant

\* Significance at 1% level

## DISCUSSION

It is observed that none of the tissues reacted positively with the control antisera, showing that there is no natural antibody in rabbit against the different tissue antigens of the castor semilooper, *A. janata*. Vg synthesis in the spinning stage larva and day 1 pupa suggested that in *A. janata*, it is triggered by endocrine events during metamorphosis. Similar results have been observed in *Hyalophora cecropia* and *Bombyx mori* (Hagedorn and Kunkel, 1979). Formation of a precipitin band with hemolymph of the female pupa and the anti-yolk serum and their absence in the larval stage, confirms the presence of Vg in pupal stage and their absence in larvae. The sequestration of Vg into hemolymph starts on day 1 pupa and continues to day 2, day 3 and day 4. Vg appears in the hemolymph 1–2 days after emergence of the adult in *Danaus plexippus* and ligation of adults shortly after emergence removes the source of JH preventing the appearance of Vg (Pan and Wyatt, 1971). In *B. mori* Vg appears in the hemolymph after the larval-pupal ecdysis (Kawaguchi and Doira, 1973) and in *H. cecropia* Vg first appears in the hemolymph shortly after the larva spin its cocoon (Hagedorn and Kunkel, 1979).

The present study has shown that the uptake of Vg into developing oocytes begin on 5 day old pupa and continues upto day 6, day 7 and day 8. In the gypsy moth, *L. dispar*, synthesis and uptake of Vg are clearly separate and independently regulated processes (Davis, *et al.*, 1990). Immunological studies have shown that Vg synthesis starts in the fat body on day 4 (spinning stage) fifth instar larva. These results clearly shows that synthesis of Vg in *A. janata* fat body appears to be part of a programmed developmental response to the metamorphic molt.

The non significant results observed by neck ligation suggests the role of some source other than JH on ovarian development. The rudimentary ovarioles and non-development of oocytes after thorax ligation suggests clearly the role of hormone of PTG on ovarian development. According to Tanaka *et al.* (1994) PTG was not required for the induction of ultranumerary level ecdyses by ecdysone and that ecdysone, or its metabolites other than ecdysterone, has a critical role in the moulting process in *B. mori*.

20-HE induced oocyte maturation in *A. janata*. The increased length of ovariole and number of oocytes/ovariole is a direct hormonal effect. 20-HE plays a major role in ovarian growth in *L. dispar* (Davis, *et al.*, 1990). The development of ovary in *B. mori* is induced by ecdysteroids, and the ovary seems to undergo progressive development accompanied by Vg accumulation (Tsuchida, *et al.*, 1987). A similar endocrine regulation may be operative in the vitellogenesis of *A. janata* since it occurs during pupal stage.

In the present study application of 20 µg of 20-HE induced completion of ovarian development and vitellogenesis during pharate adult stage suggesting that it is under the general regulation of metamorphosis. The ovarian tissues responded directly to the concentration of 20-HE and not through secondary endocrine regulators. From the present study, it is clear that the development of ovary in *A. janta* is induced by ecdysteroids, and the ovary seems to undergo progressive development that accompanies Vg accumulation.

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## Biological Control Studies on the Mango Green Shield Scale *Chloropulvinaria polygonata* (Ckll.) (Homoptera, Coccidae) in India

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**Abstract:** The green shield scale *Chloropulvinaria polygonata* (Ckll.) has become a serious pest of mango in Indian subcontinent inspite of application of insecticides. *C. polygonata* was found attacked by three parasitoids namely *Coccophagus bivittatus* Comp. *C. nigricorpus* sp. nr. *Metaphycus helvolus* sp. nr., and three predators viz. *Cryptolaemus montrouzieri* Muls., *Mallada astur* (Banks) and *Spalgis epius* Westwood in mango orchards around Bangalore. The coccinellid *C. montrouzieri* is a new record on *C. polygonata*, though it has been recorded on other species of *Chloropulvinaria*. A study conducted on the predatory potential of *C. montrouzieri* revealed that the predator consumed about 2400 eggs of *C. polygonata* in its larval developmental period of 15.40 days. *C. montrouzieri* was found very effective in suppressing the scale population in two mango orchards near Bangalore.

**Keywords:** Mango, Green shield scale, *Chloropulvinaria polygonata*, Natural enemies, Predatory potential, *Cryptolaemus montrouzieri* suppression.

### INTRODUCTION

Over 48 species of scale insects have been reported from mango in the Indian subcontinent (Butani, 1979). The green shield scale *Chloropulvinaria* (= *Pulvinaria*) *polygonata* (Cockerell) has been reported as a pest of mango in India (Srivastava *et al.*, 1989), Pakistan (Mahdihassan, 1978), Bangladesh (Ali, 1978). The scales are usually seen on the undersurface of leaves and shoots, and the scale infestation sometimes extends to flowers and fruits. They suck the cell sap resulting in the loss of vigour of mango plants. The nymphs and adults secrete honeydew which encourages the development of sooty mold (*Capnodium mangiferum*). The photosynthetic activity of such scale

infested plant is adversely affected (Chatterji and Datta, 1974). Though some insecticides have been recommended for controlling *C. polygonata* in India (Tandon and Srivastava, 1980a; Gupta and Singh, 1988; Srivastava *et al.*, 1989), it is difficult to achieve perfect control of scales with these conventional insecticides mainly due to the mealy covering over their bodies (Chatterji and Datta, 1974). Eggs of the scales, protected by waxy filamentous secretions of ovisac, are almost impossible to reach with insecticides. On the other hand, scale insects being sessile in nature, are more amenable for biological control. About 20 natural enemies have been recorded so far from *C. polygonata* in India (Sinha and Dinesh, 1984; Tandon and Srivastava, 1980b; Ali, 1964), Pakistan (Ahmad and Muzaffar, 1974), Bangladesh (Ali, 1978) and China (Huang, 1981). However, no serious attempts have been made so far to study the impact of natural enemies, especially the Australian lady bird beetle, *Cryptolaemus montrouzieri* Muls. in the suppression of *C. polygonata* on mango in India and elsewhere. The present study deals with (1) survey to know the current natural enemy complex of *C. polygonata* (2) assessment of predatory potential of the *C. montrouzieri* with a view to use it against *C. polygonata* since this predator is found effective against some other species of *Chloropulvinaria* (3) Field evaluation of the impact of *C. montrouzieri* in the suppression of the green shield scale in the mango orchards.

## MATERIALS AND METHODS

### Survey for Natural Enemies

The shoots and leaves infested with scales (*C. polygonata*) were collected from mango trees in cloth bags and brought to the laboratory. The samples were examined for the presence of predators, and then placed over the ripe pumpkins (*Cucurbita molchata* L.) in cloth walled wooden cages (20 × 20 × 30 cm). Adult parasitoids and predators that emerged from the samples were collected, preserved and sent for identification.

### Predatory potential of *C. montrouzieri*

*C. montrouzieri* was maintained on the mealybug *Planococcus citri* (Risso) infested pumpkin fruits in the laboratory at 25–28°C and 60–75% relative humidity as outlined by Chacko *et al.* (1978). Newly hatched predatory larvae (20) were confined individually in glass vials (5.5 × 2.5 cm) and supplied with known number of fresh eggs of *C. polygonata* daily. The number of eggs consumed by the predator in each larval instar was recorded daily, and later the total consumption during its larval development was calculated.

### Impact of *C. montrouzieri* on *C. polygonata*

The efficacy of *C. montrouzieri* in the suppression of the mango scale was studied at two locations.

The first field experiment was conducted on 10 year old mango variety Guruvam at the mango breeding block located at IIHR Farm. All the plants of the above variety found infested with the scales, were chosen for recording the observations. In each plant, 4 shoots, one shoot of 30 cm in each direction, were selected to record the data. The population of the scales and *C. montrouzieri* were observed at fortnightly intervals from September to December.

In another study, observations were recorded on 15 year old mango trees at the premises of State Poultry Farm located 3 km away from IIHR Farm. The activities of

the predator and scales were noticed in February and the observations were continued upto July.

## RESULTS AND DISCUSSION

### Survey for natural enemies

A total of six natural enemies *i.e.*, three parasitoids and three predators were collected from *C. polygonata* infesting mango trees. The primary parasitoids included *Metaphycus helvolus* sp. nr., *Coccophagus bivittatus* Comp. and *C. nigricorpus* sp. nr. All the above three parasitoids were not reported earlier on *C. polygonata*, though 13 parasitoids were known to attack the scale in India, Pakistan, China and Bangladesh (Table 1). *Cryptolaemus montrouzieri*, *Mallada astur* (Banks) and *Spalgis epius* Westwood were collected from *C. polygonata* in the present study. Among them, *C. montrouzieri* and *M. astur* were reported for the first time from *C. polygonata* in India and elsewhere. Though *S. epius* was not recorded earlier on *C. polygonata* in India, it was observed feeding on this mango scale in Bangladesh (Ali, 1978).

### Predatory potential of *C. montrouzieri*

*C. montrouzieri* prefers to feed on the ovisacs rather than the nymphs and adults of *Chloropulvinaria* spp. (Bartlett, 1977). In the present study, the number of scale insect eggs consumed by the first, second, third and fourth instar larvae of *C. montrouzieri* averaged 200.65, 337.50, 743.45 and 1105.00 respectively (Table 2). A single predatory larva was able to consume a total of 2387 eggs of *C. polygonata* in its larval developmental period of 15.40 days. The feeding of 3776 eggs of *Chloropulvinaria psidii* (Maskell) by a single larva of *C. montrouzieri* has also been reported earlier by Mani and Krishnamoorthy (1990).

### Impact of *C. montrouzieri* on *C. polygonata*

Though *C. montrouzieri* was observed feeding on various species of *Chloropulvinaria*, its efficacy in controlling *C. polygonata* had not been studied in India and elsewhere. In both the trials, *C. montrouzieri* proved very effective in suppressing the population of *C. polygonata* on mango. In the first study at IIHR Farm, the number of ovisacs of scales had declined from 32.43/shoot in October to negligible numbers in the first week of December (Table 3). Only *C. montrouzieri* was found feeding on the ovisacs in this trial, and no other natural enemy was observed.

In another trial conducted on the mango trees located at the premises of State Poultry Farm, both the scale and the predator (*C. montrouzieri*) were noticed in February. The population of ovisacs was reduced from 80.50 per shoot in the first week of February to 5.00 in the first week of July. The activity of *C. montrouzieri* was observed throughout the study period (Table 4). A higher population of *Cryptolaemus* (10.40 per shoot) was recorded in May. The parasitoids namely (*Coccophagus bivittatus* and *C. nigricorpus* sp. nr. were reared in negligible numbers.

The suppression of *C. polygonata* in both the present studies can mainly be attributed to the activity of *C. montrouzieri* which has also been reported effective against *Chloropulvinaria aurantii* (Ckll.) on citrus in USSR (Prokopenko, 1992), *C. floccifera*

Table 1: Natural enemies of *Chloropulvinaria polygonata*

Species	Family & Order	Locality	Reference
<i>Aneristus ceroplastae</i> How	Encyrtidae, Hymenoptera	Bangladesh	Ali (1978)
		Pakistan	Ahmad & Muzaffar (1974)
		India	Sinha & Dinesh (1984)
		China	Huang (1981)
<i>Encyrtus barbatus</i> Tim.	Encyrtidae, Hymenoptera	Pakistan	Ahmad & Muzaffar (1974)
<i>Metaphycus</i> sp.	Encyrtidae, Hymenoptera	India	Ali (1964)
<i>M. hederaceus</i> sp.nr.	Encyrtidae, Hymenoptera	India	Tandon & Srivastava (1980b)
<i>M. helvolus</i> comp.	Encyrtidae, Hymenoptera	Pakistan	Ahmad & Muzaffar (1974)
		Bangladesh	Ali (1978)
<i>M. helvolus</i> sp. n.	Encyrtidae, Hymenoptera	India	Present record
<i>Coccophagus</i> sp.	Aphelinidae, Hymenoptera	Pakistan	Ahmad & Muzaffar (1974)
<i>C. bivittatus</i> comp.	Aphelinidae, Hymenoptera	India	Present record
<i>C. chloropulvinariae</i> sp. nr.	Aphelinidae, Hymenoptera	India	Hayat (1974) (Havant, 1974)
<i>C. crenatus</i> sp. n.	Aphelinidae, Hymenoptera	China	Huang (1980)
<i>C. hawaiiensis</i> Timb.	Aphelinidae, Hymenoptera	China	Huang (1981)
<i>C. longifaciatus</i> Timb.	Aphelinidae, Hymenoptera	India	Present record
<i>C. silvestri</i> Comp.	Aphelinidae, Hymenoptera	China	Huang (1981)
<i>C. yoshidae</i> Nakay	Aphelinidae, Hymenoptera	China	Huang (1981)
<i>Microterys</i> sp.	Encyrtidae, Hymenoptera	Pakistan	Ahmad & Muzaffar (1974)
<i>Tetrastichus</i> sp.	Eulopidae, Hymenoptera	Pakistan	Ahmad & Muzaffar (1974)
<b>PREDATOR</b>			
<i>Cryptolaemus</i>			
<i>montrouzieri</i> Muls.	Coccinellidae, Coleoptera	India	Present record
<i>Scymnus coccivora</i> Ayyar	Coccinellidae, Coleoptera	Pakistan	Ahmad & Muzaffar (1974)
		India	Ali (1964)
<i>Berginus maindroni</i> sp.nr.	Coccinellidae, Coleoptera	Pakistan	Ahmad & Muzaffar (1974)
<i>Chilocorus nigrita</i> (Fab.)	Coccinellidae, Coleoptera	Pakistan	Ahmad & Muzaffar (1974)
<i>Pharoscyms</i>	Coccinellidae, Coleoptera	Pakistan	Ahmad & Muzaffar (1974)
<i>flexibilis</i> Muls.			
<i>Nephus regularis</i> Sic.	Coccinellidae, Coleoptera	India	Sinha & Dinesh (1984)
<i>Cybocephalus</i> sp.	Nitidulidae, Coleoptera	Pakistan	Ahmad & Muzaffar (1974)
<i>Chrysopa</i> sp.	Chrysopidae, Neuroptera	Pakistan	Ahmad & Muzaffar (1974)
<i>Mallada astur</i> (Banks)	Chrysopidae, Neuroptera	India	Present record
<i>Eublemma</i> sp.	Noctuidae, Lepidoptera	Pakistan	Ahmad & Muzaffar (1974)
<i>Spalgis epius</i> Westwood	Lycanidae, Lepidoptera	Bangladesh	Ali (1978)
		India	Present record

Table 2: Feeding potential and development of *Cryptolaemus montrouzieri* on *Chloropulvinaria polygonata*

Larval instar of <i>Cryptolaemus</i>	No. of <i>Chloropulvinaria</i> eggs consumed (Mean $\pm$ S. D.)	Developmental period of <i>Cryptolaemus</i> (days) (Mean $\pm$ S. D.)
I	200.65 $\pm$ 15.32	4.30 $\pm$ 0.42
II	337.50 $\pm$ 18.40	2.50 $\pm$ 0.27
III	743.45 $\pm$ 34.74	3.65 $\pm$ 0.56
IV	1105.60 $\pm$ 63.68	4.95 $\pm$ 0.50
Total	2387.00 $\pm$ 68.94	15.40 $\pm$ 2.10

Table 3: Population of *C. polygonata* and *C. montrouzieri* at IHR Farm

Date of Observation	Population/Shoot (Mean $\pm$ S.D.)	
	<i>Chloropulvinaria ovisacs</i>	<i>Cryptolaemus</i> larvae
4th October	32.43 $\pm$ 10.30	4.82 $\pm$ 3.10
20th October	27.84 $\pm$ 9.70	6.44 $\pm$ 4.26
6th November	10.57 $\pm$ 5.83	5.35 $\pm$ 3.41
21st November	4.63 $\pm$ 1.42	1.97 $\pm$ 1.65
5th December	0.72 $\pm$ 0.36	0.25 $\pm$ 0.80

Table 4: Field population of *C. polygonata* and *C. montrouzieri* on mango at State Poultry Farm

Date of Observation	Population/Shoot (Mean $\pm$ S.D.)	
	<i>Chloropulvinaria ovisacs</i>	<i>Cryptolaemus</i> larvae
4th February	80.50 $\pm$ 16.36	1.30 $\pm$ 0.92
10th March	74.30 $\pm$ 14.74	5.40 $\pm$ 1.38
14th April	81.40 $\pm$ 19.51	7.20 $\pm$ 2.40
12th May	40.30 $\pm$ 6.48	10.40 $\pm$ 5.14
8th June	15.00 $\pm$ 3.90	7.20 $\pm$ 3.35
7th July	3.00 $\pm$ 0.86	0.70 $\pm$ 0.41

(West wood) on tea in USSR (Mzhavanadae, 1984), *C. maxima* (Green) on neem in India (Tirumala Rao and David, 1958), and *C. psidii* on guava (Mani and Krishnamoorthy, 1990). It is advantageous to use this predator since it was also found efficient in suppressing the mealybug *Rastrococcus iceryoides* (Green) on mango (Mani *et al.*, 1995).

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## Response of Muga Silkworm *Antheraea assama* to Host Quality

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**Abstract:** An experiment conducted to assess the response of the Muga silkworm, *Antheraea assama* Westwood (Lepidoptera: Saturniidae) to its host quality revealed that leaf moisture content of the Som Plant, *Machilus bombycina* King (Lauraceae) regulated the rate of ingestion by the silkworm; higher the moisture content, higher the feeding rate and *vice versa*. However, dietary water, in this case, acted not as a phagostimulant but as a diluent of nutrients. Therefore, the silkworm had to ingest more, to compensate the necessary nutrients required to its growth and development. Dietary water did not stimulate growth as evident from the difference in head capsule width and growth indices of the larvae during various seasons.

**Keywords:** *Antheraea assama*, ingestion rate, dietary water, phagostimulant, growth and development.

### INTRODUCTION

Though nutritional requirements of an insect is understood qualitatively, quantitatively they are less known. As such there have been few studies on rates of food intake in insects, however, quite a few of them have conclusively established that the intake rate is very useful in demonstrating the differences in food efficiency as well as in determination of absolute requirements of various dietary constituents (Waldbauer, 1964). Excepting a few, there were more than one optimal diet for a given species in which water might act as a phagostimulant as well as diluent of nutrients (Sang, 1959; Dadd, 1960; House, 1965; Carell, *et al.*, 1990; Bardoloi and Hazarika, 1992). These observations give rise to questions concerning the efficiency of ingestion as well as behavioural and physiological control of feeding rate. Is there a direct behavioural response to increased water content in a diet? Can a diluted diet or less concentrated diet taste noticeably different to insects? This article attempts to answer these by studying the role of dietary water in influencing the consumption rate and also on the relationship of feeding rate to the growth and development of the muga silkworm, *Antheraea*

*assama* Westwood (Lepidoptera: Saturniidae), a native sericigenous insect of Assam, India (Bardoloi and Hazarika, 1992).

## MATERIALS AND METHODS

Originally disease free eggs of *A. assama* were obtained from the Central Silk Board, Jogduar Unit, Jorhat, Assam. Several generations from this stock were cultured and maintained in our laboratory. Larvae were reared in the orchard, Department of Horticulture, Assam Agricultural University, Jorhat on Som, *Machilus bombycina* (King) (Lamaceae) plants. Descriptions of host plants were given in Bardoloi and Hazarika (1995).

Five different twigs with 10 leaves in each of them were selected on a host plant and the surface area of each of the leaf from a twig was drawn and measured separately on a graph paper in mm<sup>2</sup>. Then a larva was caged by a mosquito proof net to each of the twigs in order to prevent its escape.

After 24 hours of release, the larvae were removed from the twigs and the uneaten leaf blades from each of the twigs were measured. By deducting unconsumed leaf areas from the original ones, the leaf area consumed by the larvae over 24 hours (feeding rate, area in mm<sup>2</sup>/larva/day) was determined.

The moisture content of the leaves of *M. bombycina* was estimated seasonally by following a standard method of leaf moisture estimation (AOAC, 1970, ). Similarly body water content of 5th instar (24 hours post-moult) larvae of *A. assama* was also determined seasonally by the technique of Lee (1961).

The growth rate and growth index during different seasons were determined by following Bardoloi and Hazarika (1992) and Prasad and Premchand (1980), respectively.

All data were subjected to a complete randomized design analysis of variance (ANOVA). Means were compared by least significant difference (LSD) (Gomez and Gomez, 1984) procedure ( $P = 0.05$ ). Correlation coefficient ( $r$ ) was calculated between leaf moisture content and feeding rate and body water content. Regression analysis between the former set was also carried out.

## RESULTS AND DISCUSSION

Leaf moisture content varied significantly between seasons ( $F = 14.70$ ,  $d.f. = 3, 16$ ,  $P = 0.05$ ), it being the highest during the summer (64.46%) (i.e., Table 1).

Table 1: Mean  $\pm$  SE leaf moisture content and feeding rate by the larvae of *A. assama* during different seasons

Seasons	Leaf moisture content (%)	Feeding rate: Area (mm <sup>2</sup> )/day/larva
Winter	49.75 $\pm$ 1.72 c (5)	14560.0 $\pm$ 1299.95 b (5)
Spring	58.89 $\pm$ 1.72 b (5)	20345.0 $\pm$ 1403.70 a (5)
Summer	64.46 $\pm$ 1.74 a (5)	22175.5 $\pm$ 2247.11 a (5)
Autumn	50.76 $\pm$ 1.68 c (5)	19087.0 $\pm$ 387.88 ab (5)

Means within column followed by the same letter(s) are not significantly different ( $P = 0.05$ , LSD, Gomez and Gomez, 1984). Values within parentheses indicate sample size.

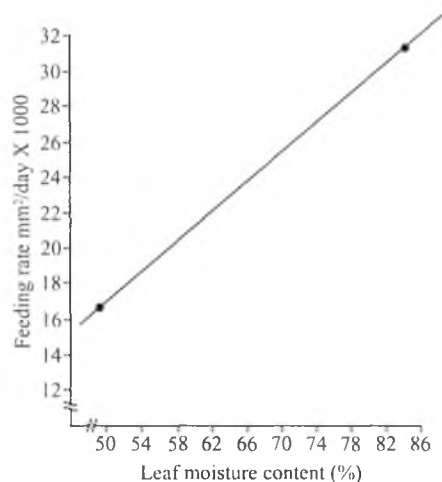


Fig. 1. Feeding rate of *A. assama* larvae as a function of leaf moisture content. Regression equation =  $-5124.18 + 431.46 x$ ,  $r^2 = 0.49$ ,  $n = 20$

Table 2: Body water content, width of head capsule and growth index of *A. assama* larvae during different seasons

Seasons	Body water content of larvae (%)	Head capsule width (mm)	Growth index
Winter	80.73±0.83 b (5)	3.97±0.51 b (4)	3.12
Spring	89.08±0.83 a (5)	4.19±0.50 a (4)	3.35
Summer	87.79±0.83 a (5)	3.86±0.52 a (4)	2.23
Autumn	82.92±0.83 b (5)	4.16±0.54 a (4)	3.94

Means within columns followed by the same letter are not significantly different ( $P=0.05$ , LSD, Gomez and Gomez, 1984). Values within parentheses indicate sample size.

Feeding rate of the larvae changed significantly during different seasons ( $F = 3.79$ ,  $d.f. = 3, 16$ ,  $P = 0.05$ ). As shown in Table 1, the highest feeding rate was recorded during summer ( $22175.5 \text{ mm}^2$ ) followed by spring ( $20345.0 \text{ mm}^2$ ) and winter ( $14560.0 \text{ mm}^2$ ). Feeding rate was positively related with the leaf moisture content ( $r = 0.70$ ,  $d.f. = 18$ ) (Fig. 1). Similar relationships between feeding rate and dietary water content were reported by House (1965), Fraenkel (1959), Waldbauer (1964). Fraenkel (1959) termed the dietary water content as a “token feeding stimulus” who showed that larvae of *Celerio euphorbiae* (L.) compensated for the low nutrient concentration by increasing the feeding rate in those host plants which were rich in water but poor in nutrients. Water in this case acted as diluents.

Body water content of the larvae was characterized by seasonal fluctuations. The 5th instar of *A. assama* of the spring and summer generations contained significantly higher water contents than those of the winter and autumn (Table 2). Leaf moisture affects the body water content of *A. assama* (Bardoloi and Hazarika, 1992) and *Lytta polita* (Carell, *et al.*, 1990). On the other hand, body water content was positively correlated with the feeding rate ( $r = 0.50$ ,  $d.f. = 18$ ).

Head capsule widths differed significantly between seasons ( $F = 27.38$ ,  $d.f. = 3$ ,  $16$ ,  $P = 0.05$ ) which were the largest during spring and autumn (4.19 and 4.16 mm, respectively; Table 2). Table 2 shows the growth index of *A. assama* during different seasons which was the lowest during summer (2.33) and the highest during autumn (3.94). The observed differences in the growth rate and growth index of *A. assama* can also be explained by House's hypothesis (1965). In our study, it was evident that although feeding rate was the highest during summer but the actual growth of the larvae was at the lowest. However, rate of biting is inversely related to body and ambient temperature (Bardoloi and Hazarika, 1995). During spring and autumn, growth was higher despite feeding rate being low. Dilution of nutrients due to higher percentage of water may be responsible for increased feeding rate. Water did not act as a phagostimulant for this insect but as a diluent of nutrients.

From the above discussion it is reasonably clear that water content in the diet is one of the major factors in limiting the feeding rate thereby growth and development of insects. However, other physical and chemical properties of food can also play important roles in determining preference by the larvae either in association with water or alone which needs further studies.

#### ACKNOWLEDGEMENTS

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## Biology of *Cotesia glomeratus* (Linnaeus) (Hymenoptera: Braconidae) A New Larval Endoparasitoid of *Pericallia ricini* Fabricius (Lepidoptera: Arctiidae)

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**Abstract:** *Cotesia glomeratus* (L) is reported for the first time as a new larval endoparasitoid of *Pericallia ricini* F. Mating and oviposition of this parasitoid takes place on the same day of emergence. The adult parasitoid prefers early stage larvae of *P. ricini* for oviposition. For a successful oviposition on *P. ricini* the adult female parasitoid takes only 4 to 5 seconds. The parasitoid usually lays only one egg in each host. The final instar parasitoid larva emerging from the host body first spins a thin semispherical dome-shaped cocoon net before spinning the usual *Apanteles* - type cocoon. The duration of immature stages from egg to final instar larval emergence of *C. glomeratus* was found to be 10 to 19 days with a mean of 11–87 days. The pupal period was completed in 5 to 6 days. Thus the total developmental period of *C. glomeratus* from egg to adult emergence was completed in 17.4 days with a range from 15 to 25 days ( $n = 18$ ) (Temp. min. 26°C; max. 30.2°C; R.H. 64%). In the field the pupae of *C. glomeratus* were parasitised by an eulophid hyperparasitoid.

**Keywords:** *Cotesia glomeratus* (L), *Pericallia ricini* F., biology, hyperparasitoid.

### INTRODUCTION

The hairy caterpillar, *Pericallia ricini* F. (Lepidoptera: Arctiidae) is a polyphagous pest and is common in Kerala, attacking banana plantations (Nair, 1986). The adult female moth lays eggs in groups of 50 to 300 on the undersurface of the banana leaves. The egg hatches in about 4 to 5 days. The larvae feed in groups voraciously on the leaf surface in its early stages and in later stages they migrate to tender leaves. Because of the voracious feeding of large number of larvae on the young banana plants the whole plant may sometimes give a burnt appearance with the whole leaves damaged and hence the vitality of the plant is also affected. The pest is reported to be parasitised by *Glyptapanteles obliquae* (Wilkinson), *Apanteles ricini* Bhat., *Meteorus* sp., *Strumia* sp., *Thelaira nigripes* F., *Euplectrus* sp. and *Henecospilus rufus* Tosq. (Nair,

1986; Ghosh, 1995). In addition to the above parasitoids, in the present paper, *Cotesia glomeratus* L. (Hymenoptera: Braconidae) is also reported for the first time as a new larval endoparasitoid of *Pericallia ricini*. Since its biology on *P. ricini* is found to differ from the biology of *A. glomeratus* already described by earlier workers, a detailed study has been undertaken on its biology and the results are presented in this paper.

## MATERIALS AND METHODS

### Rearing of the host

A culture of *P. ricini* was maintained in the laboratory on the larvae collected from the field. These larvae were grown on banana leaves in specimen jars of size 20 × 10 cms. When these larvae pupated they were transferred to another jar of size 15 × 10 cms. The adults emerging from these pupae were liberated into cages of size 35 × 35 × 35 cms, each with a wooden base forming the floor, with wire nets on three sides and with glass on the other two opposite sides. The eggs laid by these moths were studied and on hatching the first instar larvae were transferred to another jar (20 × 10 cms) and were fed on banana leaves.

### Rearing of the parasitoid

Cocoons of *C. glomeratus* collected from the field were put in glass tubes of size 15 × 2.5 cms. The adult parasitoid which emerged from these cocoons were fed on 50% honey. Laboratory reared I and II instar larvae of *P. ricini* were provided to the mated females of *C. glomeratus* for oviposition. The oviposited larvae were transferred to glass tubes of size 15 × 2.5 cms containing tender banana leaves.

All rearing works were conducted under the laboratory conditions. (Temp. min. 26°C, max. 30.2°C, and with R.H. 64%).

## RESULTS AND DISCUSSION

*Cotesia glomeratus* (L) also known by the name *Apanteles glomeratus* (L) is a common and wide spread parasitoid which attacks a wide variety of lepidopterans, including *Pieris brassicae* (Walker, 1995). The species of *A. glomeratus* has been reported from India, Russia, England, Denmark, France, Spain, Germany, Norway, Holland, Africa, Cyprus and U.S.A. In Punjab (India) *P. brassicae* is reported to be parasitised by *A. glomeratus* (Rataul, 1976). In Kerala *C. glomeratus* (*A. glomeratus*) is found to parasitise the hairy caterpillar, *P. ricini* (Fig. 1a) of banana. In *P. ricini*, the parasitoid *C. glomeratus* develops as a solitary endoparasitoid.

### Life History

The adults of the parasitoid *C. glomeratus* emerge from the cocoon by cutting a circular lid at one end of the cocoon (Fig. 1c & d). Mating and oviposition of the parasitoid takes place on the same day of emergence. The adult parasitoid prefers early stage larvae of *P. ricini* for oviposition (Fig. 1b). For a successful oviposition on the host *P. ricini*, a female parasitoid of *C. glomeratus* took only 4 to 5 seconds. Rataul (1976) has reported that *A. glomeratus* took 20 to 40 seconds for oviposition on young caterpillars of *P. brassicae*. In old caterpillars, the time was reported to exceed 50 seconds. According to Hamilton (1935-36) *A. glomeratus* took an average of 20.4 seconds for

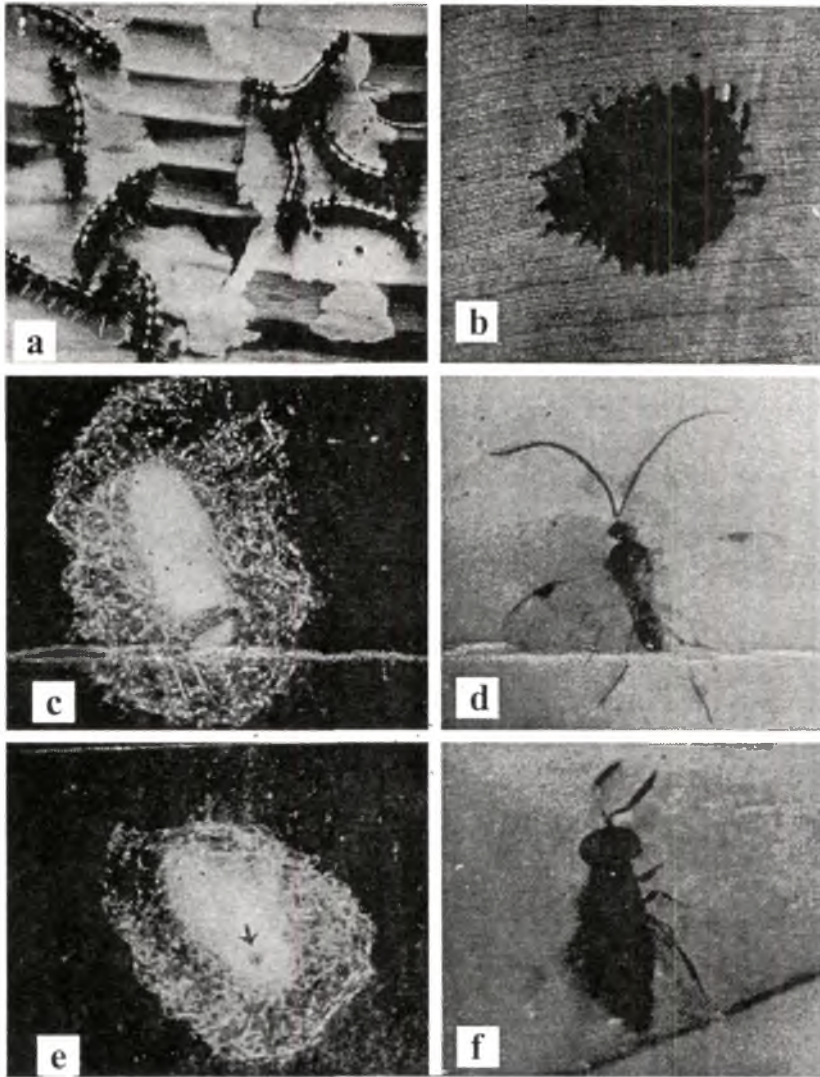


Fig. (1a) *Pericallia ricini* larvae feeding on banana leaves; Fig. (1b) First instar larva of *P. ricini* (susceptible stage of *C. glomeratus*); Fig. (1c) Cocoon of *C. glomeratus*; Fig. (1d) Adult of *C. glomeratus*; Fig. (1e) Hyperparasitoid exit hole on the cocoon of *C. glomeratus*; Fig. (1f) Hyperparasitoid of *C. glomeratus*.

oviposition within a range of 13 to 30 seconds. In the above cases, Rataul (1976) and Hamilton (1935-36) reported *A. glomeratus* as a gregarious parasitoid laying several eggs in a single host. The adult of the parasitoid *C. glomeratus* laid only one egg in each *P. ricini* larva and if 2 to 3 eggs were laid, only one develops into the adult stage. Thus *C. glomeratus* is a solitary endoparasitoid of *P. ricini*. However *A. glomeratus* laid an average of 28.6 eggs on a single host of *P. brassicae* (Rataul, 1976) Hamilton (1935-36) recorded an average of 30.5 eggs and Gatenby (1919) recorded 30 to 60

eggs in the body cavity of a single host.

The parasitised host larva leads a normal life at the early stage of parasitisation and feeds on the banana leaf. As the developing parasitoid larva feeds on the internal tissues of the host caterpillar, the host larva becomes sluggish. The final instar larva of the parasitoid comes out of the host caterpillar by cutting the host body wall with the help of its mandibles. The parasitoid larva emerges completely from the host body and spins a cocoon usually on the leaf of the banana. *Glyptapanteles obliquae* a gregarious parasitoid of *P. ricini* is reported to spin the cocoon even before the complete emergence of parasitoid larva from the host body (Ghosh, 1995). In *G. obliquae* the last larval moulting takes place at the time of its emergence from the host body and the exuvia is pushed back slowly from the body of the parasitoid larva so that it gets plugged in the emergence hole (Ghosh, 1995). Since the last larval instar of *G. obliquae* is with well developed and well sclerotised mandibles, the internal tissues of the host larva and the other developing parasitoid larvae found in the body cavity of the host are protected from damage by the delayed moult of the last larval instar. It is an important parasitoid adaptation of *G. obliquae* evolved for gregarious life. Such an adaptation is not generally found in solitary parasitoids as reported in *A. taragamae* (Ghosh, 1987). The latter was found to feed on the host tissues, even after the emergence from the host body.

Before spinning a cylindrical rod-shaped *Apanteles* - type cocoon the parasitoid larva of *C. glomeratus* spins a thin semispherical dome-shaped cocoon net (about 6.9 mm in length, 5.03 mm in breadth and 4.2 mm in height). This semispherical cocoon net is spun very fast by the emerged final instar larva of *C. glomeratus* so as to hide it from the attack of hyperparasitoids, as it is highly susceptible to the attack of hyperparasitoids. By concealing within this cocoon net the parasitoid larva spins the usual *Apanteles* - type cylindrical rod-shaped cocoon with a length of 4.25 mm and breadth of 1.5 mm. The cocoon spinning is completed in 3.5 to 4 hours. The cocoon of *C. glomeratus* is slightly yellowish white in colour.

When *P. ricini* larvae were parasitised by *Glyptapanteles obliquae*, the host larva remains alive till the emergence of the adult parasitoid from the cocoon, or sometimes even after the exit of parasitoid adults. The host caterpillar (*P. ricini*) from which *G. obliquae* larvae emerged shows wriggling movements of the head when slightly disturbed. This behaviour of the host caterpillar is reported to protect the cocoon of the parasitoid *G. obliquae* from the attack of hyperparasitoids (Ghosh, 1995). Moreover, *G. obliquae* is found to spin the cocoon below the host larva (*i.e.*, on its undersurface) where as *C. glomeratus* larva usually spins the cocoon away from the host. Thus *C. glomeratus* cocoon is more prone to the attack of hyperparasitoids. As a result it has developed the behaviour of spinning a cocoon dome initially. The adults of *C. glomeratus* emerge from the cocoon by cutting a circular lid at one end of the cocoon (Fig. 1c & 1d).

The duration of immature of stages (Table 1), from egg to final instar larval emergence, of *C. glomeratus* is 10 to 19 days with a mean of 11.8 days ( $n = 18$ ). The pupal period is completed in 5 to 6 days with a mean of 5.37 days ( $n = 15$ ). Thus the total developmental period of *C. glomeratus* from egg to adult emergence is completed in 17.4 days within a range of 15 to 25 days ( $n = 18$ ) (temp. min 26°C; max. 30.2°C; R.H. 64%).

Table 1: Lifecycle of *Cotesia glomeratus* L.

Stage	Duration in Days		
	Min.	Max.	Average $\pm$ SD
Endoparasitic stage (egg + larval duration)	10	19	11.87 $\pm$ 2.9
Pre-pupal + pupal duration	05	06	5.37 $\pm$ 0.48
Total duration of immature stages	15	25	17.4 $\pm$ 2.5

In the laboratory, adults of *C. glomeratus* were found to live for 7 to 9 days when fed with 50% honey. When provided with flower and resin, adults of *A. glomeratus* were reported to live for a day in the laboratory (Rataul, 1976).

### Hyperparasitoid

In the field, a eulophid hyperparasitoid (Fig. 1f) is found to attack the pupae of *C. glomeratus*. The hyperparasitoid first enters the cocoon net and inserts the ovipositor into the pupa through the cocoon. Up to 10 eggs were laid by a female hyperparasitoid in a single *C. glomeratus* pupa. The immature stages of the hyperparasitoid were found to complete their development in 16 days ( $n = 12$ ) (temp. min. 26°C; max. 30.2°C; R.H.64%). All the adults of the hyperparasitoid developed from one pupa of *C. glomeratus* usually emerge through a single hole, made on the cocoon (Fig. 1e).

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## Suppression of *Ludwigia adscendens* by Naturally Occurring Biocontrol Agents in Bangalore, India

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**Abstract:** Studies under field conditions in Bangalore, revealed that the aquatic weed *Ludwigia adscendens* is kept under check by the combined action of a leaf feeder *Altica caerula* (Coleoptera: Chrysomelidae) and a pod borer *Nanophyes nigrutilus* (Coleoptera: Curculionidae), which affect the vegetative and reproductive potential of the weed. Releases of these insects is likely to result in successful biological control of *L. adscendens* and related weeds in other parts of India and in other countries of South East Asia.

**Keywords:** *Ludwigia adscendens*, *Altica caerula*, *Nanophyes nigrutilus* Biological control.

### INTRODUCTION

The water primrose *Ludwigia adscendens* (L.) Hara (Onagraceae) and its related species are considered as major weeds in rice fields and fresh water-bodies in India (Gupta, 1979). *L. adscendens* is also reported as a common weed in rice fields of Thailand, Taiwan, Japan, Korea and Malaysia. (Institutum Botanicum Academia Sinicae, 1972; Kims, 1982; Tang Hong Yuam, *et al.*, 1988; Mou-Yen Chiang, 1992). Preliminary observations showed presence of a chrysomelid beetle *Altica caerula* Olivier and a curculionid *Nanophyes nigrutilus* Boh. feeding on the weed in and around Bangalore. A detailed study was carried out on their seasonal abundance and potentiality in suppressing the weed under field conditions in Bangalore.

### MATERIALS AND METHODS

A fresh water lake covering 2 hectares, about 70% of which was infested by *L. adscendens*, located near the experimental farm of Indian Institute of Horticultural Research, was selected as the study site.

Twenty five random samples of *L. adscendens* twigs of 25 cms. length were collected at monthly intervals and brought to the laboratory, in separate polythene covers. Observations were made from individual samples on the total number of leaves, number of leaves damaged by the insects and the percentage of leaf damage, which was

Table 1: Effect of *A. caerulea* on the weed

Sl. No.	Month of coll.	Total leaves	% damaged			% damage	No. of insects collected		
			Low	Med.	High		Eggs	Larvae	Adults
1	June	160	20.62	20.00	12.50	53.12	0	8	0
2	July	156	12.82	21.15	25.64	19.61	0	11	0
3	Aug.	127	14.17	12.60	44.88	71.05	0	23	0
4	Sept.	0	0	0	0	0	0	0	0
5	Oct.	179	15.08	2.79	6.70	24.57	0	7	0
6	Nov.	169	14.20	2.96	6.20	23.36	15	12	7
7	Dec.	152	16.45	4.61	7.82	28.87	42	10	0
8	Jan.	150	19.33	3.33	13.33	35.99	27	15	0
9	Feb.	155	15.48	6.45	12.90	34.83	25	20	0
10	March	152	12.50	6.58	19.08	38.16	12	23	0
11	April	162	8.02	7.41	32.10	47.53	13	19	0
12	May	66	17.47	9.60	19.40	46.11	13	11	2

categorised as low (0–25%), medium (25–60%) and high (60–100%), based on visual observation. In addition, the number of eggs, larvae, pupae and adults present were also recorded.

For determining the effect of *N. nigrifolius*, the field collected buds, flowers and pods were placed in separate glass vials ( $7.5 \times 2$  cms) and plugged with cotton to prevent escape of the emerged adults. After recording the number of adults emerged the pods were cut open to study the impact of the insects on fruit setting.

## RESULTS AND DISCUSSION

### Effect of *A. caerulea* on the Vegetative Potential of the Weed

*A. caerulea* females lay creamy white eggs in clusters on the under surface of the leaves. The early instar larvae feed by scraping the leaf tissue from both surfaces, while the later instars feed on the leaves and tender portions of the stem. Under severe infestation the combined feeding of the adults and larvae causes skeletonisation of the entire weed mat.

*A. caerulea* could be collected throughout the year at Bangalore, where the mean minimum and maximum temperatures were found to range from  $33.00$ – $13.35^{\circ}\text{C}$ . It was found to cause a maximum of 71.05% leaf damage in August, of which 44.88% were fully skeletonized (Table 1). The damage was found to increase rapidly resulting in complete defoliation of the weed in the infested area, which explains the absence of insects in September. Though the attacked plants were able to put forth new leaves, the insect also appeared simultaneously. With the growth of the plant, the population of the insect also started increasing, thereby bringing the weed under heavy stress in April (Table 1).

### Effect of *N. nigrifolius* on the Reproductive Potential of the Weed

*N. nigrifolius* lay their eggs singly on the basal portion of the flowers. The development of the larvae cause rapid proliferation of the cells, resulting in malformed pods. The insects were found to cause pod damage throughout the year at Bangalore. The insect

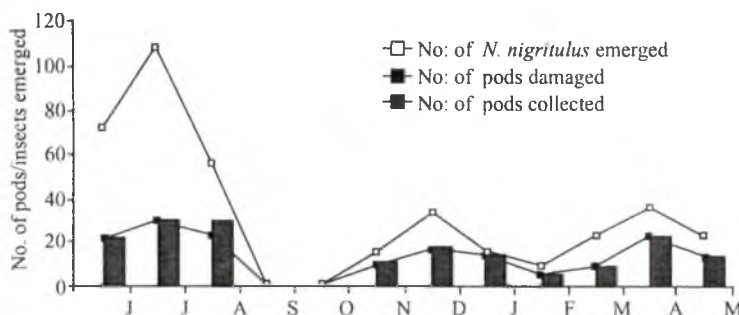


Fig. 1. Impact of *N. nigrifolius* on the reproductive potential of *L. adscendens*

could not be collected during September due to the non-availability of the pods at the study site. All pods collected during the study period were infested, except in August, when 76.66% infestation was observed (Fig. 1). The mean number of adults per pod was four. Healthy pods of *L. adscendens* were found to produce an average of 68.5 seeds. But no viable seeds could be recovered from pods infested by *N. nigrifolius*. Upto 52% of the larvae of this insect was found to be parasitized by *Tetrastichus* sp. However, parasitism was not found to reduce the effectiveness of this natural enemy, as pods from which parasites emerged also showed absence of viable seeds.

The above observations clearly demonstrate that the indigenous natural enemies *A. caerulea* and *N. nigrifolius*, effectively suppress the vegetative and reproductive potential respectively of *L. adscendens* in Bangalore. Dubey (1981) and Napompeth (1994) have reported successful control of *L. perennis* and *L. adscendens* in paddy fields by releasing *A. cynea* (Weber) and *A. foveicollis* Jacoby respectively. The present study indicates that releases of *A. caerulea* and *N. nigrifolius* could bring about successful biological control of *L. adscendens* and related species in other parts of India and other countries of South East Asia.

#### ACKNOWLEDGEMENTS

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## Influence of Host Plants on the Biological Parameters of *Aphis craccivora* Koch (Homoptera : Aphididae)

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**Abstract:** Studies on biological parameters of *Aphis craccivora* Koch at  $20\pm 1^{\circ}\text{C}$ , 50% RH and LD 16: 8 hrs photoperiod revealed the presence of four instars. Nymphal period on cowpea (*Vigna unguiculata* (Linn.) Walp.), lablab (*Lablab niger* Medik.) and pigeonpea (*Cajanus cajan* (Linn.) Mant.) was completed in 13 - 16, 13 - 16 and 14-16 days in vegetative stage and 12 - 13, 13 - 15 and 13 -14 days in flowering stage, respectively. Adult longevity in flowering stage (15.8 - 17.1 days) was lower than in vegetative stage (18.9 - 20.3 days). Similar trend was observed with respect to total life span, which was 29.1 - 30.6 days in flowering stage and 33.6 - 33.9 in vegetative stage. Shorter adult longevity, longer nymphal period and lower fecundity was observed on lablab; while pigeonpea was the least preferred host plant. The higher fecundity, adult longevity and reproductive rate of *A. craccivora* when reared on cowpea lead to its selection as the most favourable host for aphid multiplication in the laboratory. A cage was devised which could be utilised for studying the biological parameters of *A. craccivora* on different host plants.

**Keywords:** *Aphis craccivora*, biological parameters, host plants, rearing

### INTRODUCTION

Identification of appropriate laboratory host and its stage is a pre-requisite for any mass rearing programme and a continuous supply of host plants of the right species and physiological condition is one of the principal requirements for rearing of aphid hosts (Blackman, 1988) which facilitates faster development and higher reproductive rate. Therefore studies on biological parameters of aphids on different hosts have an added significance in laboratory rearing of aphids. Though it is a known fact that the type of host plant plays an important role in the multiplication of the pest population, the work on the life cycle of *Aphis craccivora* Koch in relation to laboratory rearing on different host plants and their physiological stages is scanty. Keeping in view the above facts, an effort was made to study the possible role of plant species and their

vegetative and flowering stages in influencing development of *A. craccivora*, so that the most suitable host plant could be selected for aphid multiplication in the laboratory.

## MATERIALS AND METHODS

Biological parameters of *A. craccivora* were studied on cowpea (*Vigna unguiculata* (Linn.) Walp.) var. C 152, lablab (*Lablab niger* Medik.) var. Hebbal aware and pigeonpea (*Cajanus cajan* (Linn.) Mant.) var. ES 90. The host plant testing was done in a cage which was a modification of the one suggested by Blackman (1988). A polyethylene box measuring 10 × 6.5 × 3 cm with a lid was used for making the cage (Fig. 1). The lower narrow side of the lid was cut off in order to make the lid slide over the box. The sliding lid was also provided with a wire mesh for ventilation (2 × 4.5 cm). Utilising a plastic piece with a central hole (5 mm), the inner chamber of the box was divided into two portions - an upper larger compartment (7.5 × 6.5 × 3 cm) and a lower smaller one (2.5 × 6.5 × 3 cm). The lower compartment was filled with a piece of sponge. An excised twig was placed in the box with its one end passing through the hole in the partition (Fig. 1). The sponge piece was kept continuously moist by placing the box in a container (10 cm diameter and 4.5 cm height) with a hole cut in the base. These containers were stood in water in aluminium trays which prevented the twig from drying up. Optimum level of water was maintained in the tray to avoid flooding of cages.

Twigs with two leaves and an apical bud were selected for the vegetative stage and those with flowering buds were utilised for flowering stage. The whole setup, consisting of a tray with five cages (Fig. 2), was placed in BOD incubators set at 20±1°C, 50% RH and LD 16 : 8 hrs photoperiod as recommended by Forbes *et al.* (1984) for aphid rearing. The test insects used in the present study were collected from the nethouse where *A. craccivora* was continuously reared on potted plants of cowpea, lablab and pigeonpea. Five gravid females were released into each cage consisting of the test plant at the desired stage. Each treatment was replicated ten times. The cages were observed daily for the number of progeny laid, for recording moulting and for measuring duration of instars. The total progeny count was taken for the entire period. The number of progeny produced was removed daily with a camel hair brush. The twigs were replaced as and when needed. The period during which a given aphid continued to reproduce constituted its reproductive period. The period between the date of birth of the last young one and the date of death of the female was considered as the post-reproductive period of an aphid. The adult longevity was calculated by adding pre-reproductive, reproductive and post-reproductive periods. Fecundity was measured in terms of the total number of nymphs produced by a given aphid during its life time. Data thus collected was subjected to the two way factorial RBD analysis.

## RESULTS AND DISCUSSION

The cage devised for present study keeps the cut twigs fresh for a longer period (two weeks) in comparison to the general methods followed earlier which involves wrapping the cut ends with moist cotton plug. The cage is particularly convenient as it reduces number of handling, prevents escape of the test insects and allows close observation of insects. The results obtained by using this cage are as under.



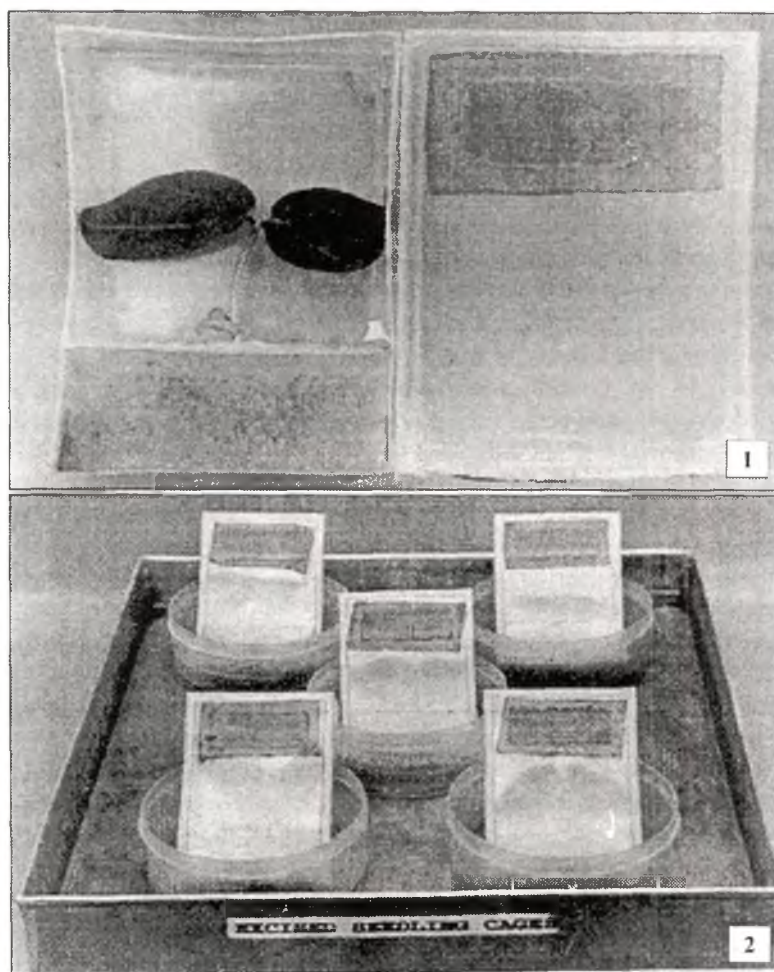


Fig. 1. Excised seedling (ES) cage utilised for studying the biological parameters of aphids

Fig. 2. Experimental set up comprising of five ES cages

The sexual life cycle of *A. craccivora* was not evident and no oviparous females were observed. The aphid reproduced only through viviparous parthenogenesis on all the crop plants tested. There are studies on the occurrence of the sexual forms of *A. craccivora* in other countries like Oklahoma (Sanborn, 1918; Grigorov, 1960; Radke, *et al.*, 1972). As early as 1918, (Das, 1918) had expressed doubts regarding the existence of sexual forms in India. However induction of sexual forms was possible in the laboratory, at 12.8°C and photoperiod ranging from 6 to 18 hours, in India (Radke, 1981). It was observed that the size and colour of the aphid differed greatly depending on the host plants. On cowpea and lablab, adults were larger and black in colour but on pigeonpea they were smaller and reddish-brown in colour. The significant effect of season and host plant on colour and size of the aphid was also observed by Hamid *et al.* (1977). Table 1 depicts the biological parameters of *A. craccivora* when reared on



different host plants at different physiological stages.

Four nymphal instars were observed on both the vegetative and flowering stage of cowpea, lablab and pigeonpea which was in conformity to the observations made by Verma *et al.* (1983). However, Bakhetia and Sidhu (1977) and Hamid *et al.* (1977) reported 4-5 moults in bean aphid. Total nymphal period was completed in 13-16, 13-16 and 14-16 days in vegetative and 12-13, 13-15 and 13-14 days in flowering stage of cowpea, lablab and pigeonpea, respectively. Shrikanth and Lakundi (1988) had observed nymphal durations of 4.84 and 5.67 days on similar varieties of cowpea and lablab, respectively. These differences can be, perhaps, attributed to variation in the temperature.

Irrespective of the stage when the host plants were compared, no significant differences were observed with reference to pre - or post - reproductive periods (Table 1). But the stage of the plant played a significant role in affecting these two parameters. Both the plant species and the stage were found to affect reproductive period of aphid significantly. Reproductive period and adult longevity was longer on cowpea (12.5 and 18.7) but nymphal period lasted longer on lablab (14.0) and pigeonpea (14.05). Total life cycle on different host plants did not differ significantly. Irrespective of the host plant, it was observed that the total life cycle was significantly longer in vegetative stage in comparison to flowering stage. However, fecundity and rate of reproduction was higher on flowering stage of plants. Both fecundity (66.1 - 65.2) and longevity (17.1 - 20.3) were significantly higher on cowpea irrespective of crop stage. Differential responses of the aphid, *Brevicoryne brassicae* L. have been reported on various hosts by Kashyap and Sharma (1994). Verma *et al.* (1983) had reported pigeonpea to be totally unsuitable for the natural breeding of aphid resulting in cessation of development beyond second or third instar. This could have been due to the variety (Ujjain 7) used by them. In the present study pigeonpea variety ES 90 proved to be the least preferred when compared to cowpea and lablab but normal development of aphids occurred on this host plant.

From amongst the various hosts tested, Waghmare and Pokharkar (1974) reported cowpea as the most preferred host of the bean aphid. In the present investigations also cowpea was identified as the most favourable host for multiplication of *A. craccivora* in the laboratory by virtue of higher fecundity, adult longevity and reproductive rate, followed by lablab.

The cage which was fabricated utilising indigenous materials could be used for similar studies on other aphid species and other sucking pests like whiteflies and mites.

## ACKNOWLEDGEMENTS

Authors are grateful to Dr. S. P. Singh, Project Director, Project Directorate of Biological Control for providing facilities and to Mr. T. V. Bhaskaran, Technical Assistant for help rendered during the investigations.

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Table 1: Biological performance of *Aphis craccivora* Koch on cowpea, lablab and pigeon pea during vegetative and flowering state

Biological characters	Crop stage	Crop			Mean
		Cowpea	Lablab	Pigeon pea	
Pre-reproductive period (Days)	Vs*	3.1	3.5	3.7	3.4
	Fs*	3.1	2.3	2.7	2.7
	Mean	3.1	2.9	3.2	
		Crop	Stage	Interaction	
	SEm	0.12	0.09	0.17	
	CD 5%	NS	0.27	0.47	
Reproductive period (Days)	Vs	13.5	13.1	12.0	12.87
	Fs	11.5	10.1	10.1	10.93
	Mean	12.5	12.14	11.05	
		Crop	Stage	Interaction	
	SEm	0.21	0.16	0.29	
	CD 5%	0.59	0.48	NS	
Post-reproductive period (Days)	Vs	3.7	3.1	3.2	3.33
	Fs	2.5	3.1	3.0	2.87
	Mean	3.1	3.1	3.1	
		Crop	Stage	Interaction	
	SEm	0.12	0.09	0.17	
	CD 5%	Ns	0.28	0.49	
Adult longevity (Days)	Vs	20.3	19.6	18.9	19.6
	Fs	17.1	16.6	15.8	16.5
	Mean	18.7	18.14	17.4	
		Crop	Stage	Interaction	
	SEm	0.25	0.21	0.36	
	CD 5%	0.71	0.58	NS	
Nymphal period (Days)	Vs	13.6	14.0	14.8	14.1
	Fs	12.7	14.0	13.3	13.3
	Mean	13.2	14.0	14.05	
		Crop	Stage	Interaction	
	SEm	0.19	0.15	0.27	
	CD 5%	0.53	0.43	0.75	
Total life cycle (Days)	Vs	33.9	33.6	33.7	33.7
	Fs	29.8	30.6	29.1	29.8
	Mean	31.9	32.1	31.4	
		Crop	Stage	Interaction	
	SEm	0.28	0.23	0.39	
	CD 5%	Ns	0.64	NS	
Fecundity	Vs	65.2	53.5	47.4	55.4
	Fs	66.1	57.6	48.9	57.5
	Mean	65.7	55.6	48.2	
		Crop	Stage	Interaction	
	SEm	0.59	0.49	0.84	
	CD 5%	1.69	1.38	NS	
Reproductive rate (Nymph/Female/Day)	Vs	4.85	4.11	4.01	4.32
	Fs	5.76	5.16	4.86	5.26
	Mean	5.31	4.63	4.44	
		Crop	Stage	Interaction	
	SEm	0.09	0.07	0.12	
	CD 5%	0.26	0.21	NS	

\* Vs: Vegetative Stage

Fs: Flowering State

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## Effect of Juvenile Hormones on the Respiration and Water Loss of the Larva of Rice Moth *Corcyra cephalonica* (Staination)

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**Abstract:** The effect of juvenile hormone on the oxygen consumption and water loss in the last instar larva of *Corcyra cephalonica* suggested that JH-I, JH-II and JH-III have an accelerating effect on the oxygen consumption. While the effect of these hormones may not or may be on the reduction of oxygen consumption in previous instars *i.e.*, I–V. The respiratory metabolism and water loss manifesting the cyclical changes of growth and development shows that the JH-I, JH-II and JH-III have inhibitory effect on I–V instars and accelerating effect on VI instar. It indicates that control of *Corcyra* with JH could be effected only when it is applied to just emerged VI instar larvae.

**Keywords:** Juvenile hormone, respiration, oxygen consumption, *Corcyra cephalonica*

### INTRODUCTION

It is discovered that juvenile hormones have specific toxicity to insects and they are not toxic to other forms of life (Williams, 1967). The toxicity of juvenoids bring about complex and integrated physiological effects on the whole population (Slama, *et al.*, 1974). One of them is respiratory metabolism. Novak (1962) demonstrated that implantation of active corpora allata increased the oxygen consumption in female in which the ovaries were functional but it did not do so in ovariectomised females. Deb and Chakravorty (1981) found that the ovaries of JH treated individuals of *Corcyra cephalonica* showed abnormal overgrowth of the mature oocytes *i.e.*, the juvenile hormone has an indirect on the total metabolism which is dependent on the stimulation of the metabolic processes in particular tissues. Similarly there is a relationship between the oxygen consumption and the water loss in different developmental stages of insects. Hence the effect of juvenile hormones on the oxygen consumption and water loss in the last instar larva of *Corcyra cephalonica* was studied.

## MATERIALS AND METHODS

*Corcyra cephalonica* larvae were reared in half dust jowar food (Theotia and Singh, 1975). The studies were carried out on II, III, IV, V and VI instar larvae. The oxygen consumption of larvae was measured by Warburg's respirometer using standard manometric techniques (Umbreit, *et al.*, 1957). For measuring oxygen consumption of II instar 20-25 larvae were weighed together and introduced in one flask. For III, IV, V and VI instar, 10, 5, 2 and 2 larvae were taken respectively.

To measure water loss the larvae were taken from the culture and allowed to starve for 24 hours.

So as to study effects of JHS on oxygen consumption and water loss the oxygen consumption of the untreated larvae was measured daily from 14th day of development from egg laying to just before pupation to obtain respiratory cycle. The water loss of II, III, IV, V and VI instar larvae was measured on 16th, 26th, 34th, 41st and 46th day.

All the larval instars were treated with juvenile hormones *i.e.*, JH-I, JH-II and JH-III. (Sigma Chemicals USA) separately. JH-I and JH-II were diluted with acetone as 1  $\mu\text{g}/\text{ul}$ . JH-III was diluted with acetone as 10  $\mu\text{g}/\text{ul}$  due to low juvenilizing activity than JH-I and JH-II (Lucher and Lanzrein, 1975).

The hormones were applied topically on abdomen with the microsyringe. The hormones were applied on the 16th, 26th, 34th, 41st and 46th day so as to measure oxygen consumption and water loss, in ten different batches and the averages were calculated.

In addition twenty larvae of last instar were taken from the culture and treated with JH-I on one day after their emergence from the fifth moult (*i.e.*, on 44th day of development) and 3 days after their emergence from the fifth moult (*i.e.*, on 46th day of development).

## RESULTS

### Oxygen consumption of normal larvae

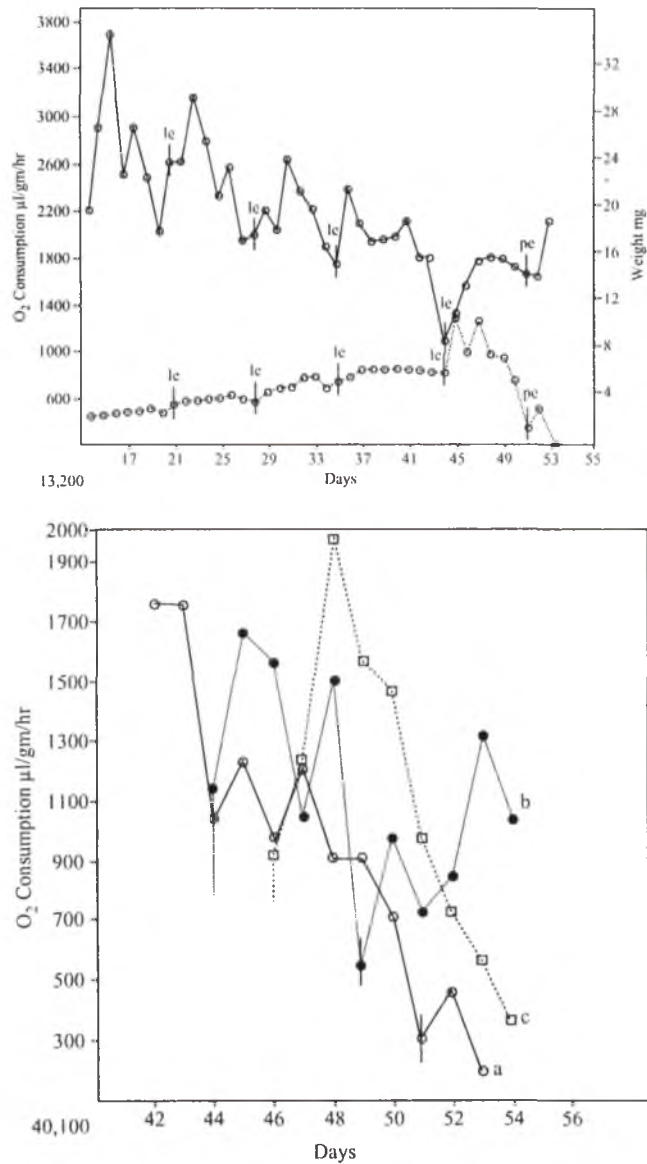
The daily readings of oxygen consumption of *Corcyra* larvae from 14th day *i.e.*, II instar onwards during the normal development show the rise of oxygen consumption in the beginning of each instar and there is decrease in the second half of the instar just before moulting; the oxygen consumption is minimum. There is subsequent decrease as the larva proceeds in its development (Fig. 1).

### Consumption of JH treated larvae

The oxygen consumption of JH-I, JH-II and JH-III treated II, III, IV, V and VI instar larvae were as shown in Table 1. These results showed that oxygen consumption of JH-treated larvae decreased as compared to normal. But in the last instar (VI) it increases with JH (Fig. 2).

### Water loss in the larvae

The rate of water loss ( $\text{mg}/\text{gm}/\text{hr}$ ) was calculated in the normal as well as in JH treated larvae and the results obtained are as in Table 2.



## DISCUSSION

The results of oxygen consumption of normal *Corcyra cephalonica* larvae show that oxygen consumption increases at the beginning and decreases towards the end of each instar *i.e.*, it is cyclical in accordance with the cycles of growth and development (Slama, 1965).

The results of oxygen consumption of juvenile hormone treated larvae show that JH-I and possibly JH-II and JH-III have a pronounced effect of increasing oxygen

Table 1: Oxygen consumption of normal JH-I, JH-II, JH-III treated larvae of *Corcyra cephalonica* staint. On 16th, 26th, 41st and 46th day from hatching

Qb. No	Larval instar	No. of days from hatching	Oxygen consumption - $\mu\text{l/gm/hr}$			
			Normal larvae	JH-I treated larvae	JH-II treated larvae	JH-III treated larvae
1	II	16	379	2627	3138	2921
2	III	26	2565	2150	2009	1997
3	IV	34	1871	699	928	1059
4	V	41	2072	908	930	972
5	VI	46	970	1564	1397	1412

Table 2: Rate of Water Loss (mg/gm/hr) of different larval instars of *Corcyra cephalonica* staint with various treatment of juvenile hormones

Qb. No	Larval instar	No. of days from hatching	Rate of Water loss mg/gm/hr			
			Untreated larvae	JH-I treated larvae	JH-II treated larvae	JH-III treated larvae
1	II	16	44.80	37.40	26.90	27.36
2	III	26	25.55	16.22	17.12	17.89
3	IV	34	20.15	16.87	16.55	16.68
4	V	41	08.9	08.05	07.29	06.72
5	VI	46	01.88	04.22	04.34	05.59

consumption of sixth instar larvae, but there may not be any effect or it may be reduced in previous (I–V) instars. This may be due to the fact that juvenilizing activity of JH-s depends on the stage of development at the time of application (Lanzrein, 1979). Lanzrein has explained it on the basis that different target tissues have their own stage specific activities to JH-s. He also suggests that metamorphosis is a sequence of events which are each dependent on the absence of JH. The decreased oxygen consumption with JH which we have obtained in earlier instars tallies with that shown by Sorensen *et al.*, (1977) in his graphs of oxygen consumption of JH (methoprene) treated larvae of *Spodoptera littoralis* Bois.

The last instar larvae treated with JH-I on 1st day after ecdysis shows extra larval instar showing cyclic oxygen consumption similar to normal larval cycle. This suggests that endogenous JH plays a role in all the instars. This can also be proved by oxygen consumption of last instar larvae, where it is seen that through there is some increase in the late JH treated larvae, the oxygen consumption follows a pattern similar to the normal larvae, *i.e.*, decreasing metabolic activity at the end of the metabolic cycle, can not be prevented even after an extra supply of the hormone (Sehnal and Slama, 1966). In case of *Dysdercus koenigii* Hebbalkar and Sharma (1991) also recorded the failure of juvenoids to induce changes in the respiratory metabolism.

Hence finally it is concluded that the increase or decrease of oxygen consumption due to JH depends on the age of the larva in a particular instar.

It is also observed that the larvae treated with JH-s shown decreased water loss than that of the normal larvae. This decreased rate of transpiration of JH treated larvae is



consequential to the decreased rate of oxygen consumption.

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## Biology of *Nesolynx dipterae* (Risbec) (Hymenoptera: Eulophidae), A New Parasitoid of *Exorista bombycis* (Louis) (Diptera: Tachinidae)

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**Abstract:** *Nesolynx dipterae* (Risbec) is gregarious ectopupal parasitoid of uzi fly *Exorista bombycis* (Louis). The adult longevity significantly increased in presence of diet and host. The adults parasitize 1 to 8 day old host however prefer young (2–4 day old) hosts. The virgin females exhibit arrhenotokous parthenogenesis and the average fecundity of gravid female was  $105 \pm 5.29$ . The net reproductive rate ( $R_0$ ), generation time (T), intrinsic rate of increase ( $r_m$ ) and the doubling time (DT) were 97.25, 21.04, 0.217 and 3.18 respectively. The population of *N. dipterae* increased 0.50 times per female per day and the sex – ratio is female biased.

**Keywords:** Biology, *Exorista bombycis*, life-table, *Nesolynx dipterae*, parasitoid.

### INTRODUCTION

Many hymenopteran parasitoids of uzi fly *E. bombycis* have been reported and are attempted to contain the uzi fly which is a serious pest of silkworm *Bombyx mori* L., causing damage to an extent of 10–20% to sericulture industry (Jolly, 1981; Narasimha Rao *et al.*, 1993). Though the biology of many parasitoids has been studied, nothing is known about *Nesolynx dipterae* (Risbec) a parasitoid recently identified from uzi fly. The biology and life-table studies of *N. dipterae* are investigated and presented in this paper.

### MATERIALS AND METHODS

Laboratory colonies of *N. dipterae* were used for the present study.

#### Longevity

Immediately after adult emergence they were transferred to 500 ml capacity conical flask @ ten females and ten males per conical flask. A total of 5 treatments *viz.* Parasitoids with host and without diet (T1), parasitoids with only host (T2), parasitoids

with only diet (T3), parasitoids with water and host (T4) and parasitoids with both diet and host (T5) were tested and each treatment was replicated five times.

Two day old uzi fly puparia and the diet containing sucrose and honey (1 : 1) were provided as per the treatments mentioned above. The survival of the parasitoid was recorded as the number of days from emergence to death.

To determine whether fertilization is required for hatching of the eggs, twenty virgin females of *N. dipterae* were separated immediately after emergence and each female was provided with 100 two days old pupae. The parasitized pupae were held for emergence of parasitoids and host adults.

### Effect of host age

One hundred healthy '0' to '10' day uzi fly puparia were provided to five pairs of one day old parasitoids in 500 ml capacity glass beakers. The exposed pupae were replaced every 24 h, and were maintained separately for emergence of parasitoid and host adults.

### Mating and Oviposition behaviour

A pair of female and male adults were confined in 1000 ml glass beaker. The sequence of pre and post mating behaviour was observed. Similarly, the ovipositional behaviour was studied by introducing five gravid females in 1000 ml glass beaker containing 25 fresh healthy host puparia.

The behaviour of gravid females from introduction to actual process of oviposition was observed.

The host puparia exposed to parasitoids for 24 h were maintained separately. A few numbers were dissected out every day, till the adult parasitoid emergence, to record the duration of egg, larva and pupal stages of the parasitoid.

### Construction of Life-table

Freshly emerged five pairs of adults were taken in 500 ml conical flask in three replications. They were provided with one to three day old twenty fresh uzi fly puparia for every 24 h till the death of the experimental adults.

The exposed puparia were maintained separately and observed for adult emergence. The number of females died on the successive days and the number of females produced per female were recorded. The life-table was prepared according to Andrewartha and Birch (1954). The intrinsic rate of increase  $r_m$  was calculated using the formula.

$$\sum_x e^{-rx} l_x m_x = 1$$

Where

$x$	=	pivotal age in days
$l_x$	=	age specific longevity
$m_x$	=	number of females produced/female
$R_0$	=	net reproductive rate
$T$	=	generation time
$DT$	=	doubling time
$r_m$	=	intrinsic rate of increase
$\lambda$	=	finite rate of increase

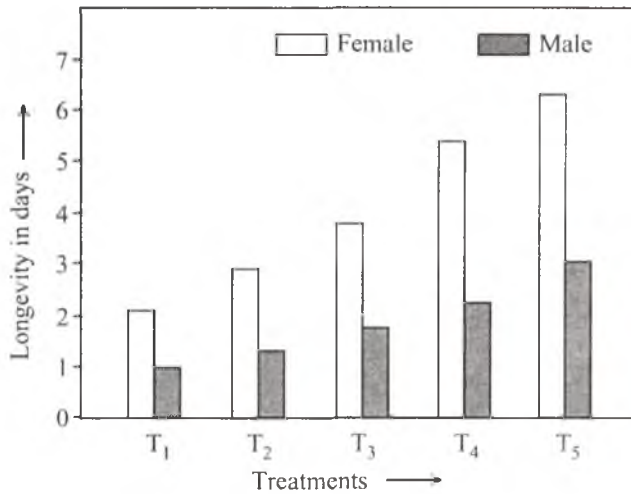


Fig. 1. Longevity of adults of *Nesolynx dipterae* (Risbec)

The net reproductive rate  $R_0$  is the number of times the population multiplies per generation and it is given by  $R_0 = \sum l_x m_x$ . The generation time  $T$ , the finite rate of increase  $\lambda$  and the doubling time  $DT$  were calculated using the formulae.

$$\begin{aligned} T &= \frac{\ln R_0}{r_m} \\ \lambda &= e^r \\ DT &= \frac{\ln 2}{r_m} \end{aligned}$$

where  $e$  is the base of natural logarithms and  $\ln$  refers to the natural logarithms.

## RESULTS AND DISCUSSION

In the present investigation the adult longevity of *N. dipterae* increased significantly in the presence of diet and host compared to other treatments (Fig. 1). Significant reduction in the life span was observed when the adults were provided with neither host nor diet. The presence of water and host slightly increased the life span. Irrespective of presence or absence of host and food, the females lived longer than males. Similar observations have been reported in *Spalangia nigra* Latrielle (Hall and Fischer, 1988), in *Antrocephalus hakonensis* (Ashmead) (Mohandas and Abdurahiman, 1992), in *Trichopria* sp, *Exoristobia philippinensis* Ashmead, *Spalangia endius* Walker (Nirmala, 1994) and also in *Nesolynx thymus* (Girault), *Pachycrepoideus veerannai* Narendran and Anil and *Dirhinus anthracia* Walker (Jyothi, 1994).

Arrhenotokous parthenogenesis was observed in *N. dipterae* as has been reported in *Tetrastichus howardi* (Olliff) (Ramkishore *et al.*, 1993), *N. thymus* (Pradip Kumar *et al.*, 1986), *Trichopria* sp, *E. philippinensis* (Veeranna *et al.*, 1987a; Veeranna *et al.*, 1987b), *S. endius* (Veeranna and Nirmala, 1992) *D. anthracia* and *P. veerannai* (Veeranna and Jyothi, 1988; Veeranna and Jyothi, 1994) which are parasitic on uzi fly pupae.

The adults of *N. dipterae* parasitized 1 to 8 days old host puparia but preferred young age puparia (Fig. 2). Similar observations have been reported in *N. thymus* (Pradip

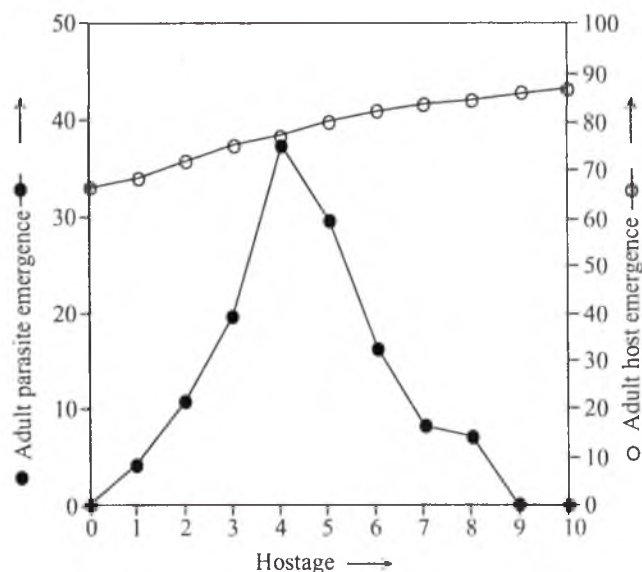


Fig. 2. Effect of hostage on parasitization of *Nesolynx dipterae* (Risbec)

Kumar *et al.*, 1986), *T. howardi* (Ramkishore *et al.*, 1993), *Trichopria* sp, *E. philippinensis* and *S. endius* (Nirmala, 1994), *D. anthracia* (Jyothi and Veeranna, 1993).

In *N. dipterae*, the adults are sexually matured on emergence and mated immediately after emergence. The males play aggressive part to establish copulation. They chase the females with fluttering of wings, and subsequently mount on them, slip backwards and copulate. The copulation lasted for 30 sec. to 1 minute. The interference of males with the mating pair is common when they are in group. These observations are in accordance with the reports on *T. howardi* (Ramkishore *et al.*, 1993), (Ramkishore *et al.*, 1993), *N. thymus* (Pradip Kumar *et al.*, 1986), *Trichopria* sp., *E. philippinensis* and *S. endius* (Nirmala, 1994).

When the host puparia were exposed to the adults of *N. dipterae* repeated probing of the host was observed which may result in the selection of suitable site for oviposition. The duration of different developmental stages is given in Table 1. Similar to *N. thymus* an Eulophid parasitic on uzi fly, *N. dipterae* is also an ectoparasitic gregarious parasitoid of uzi fly. The average fecundity recorded in the laboratory was  $105.0 \pm 5.29$ , and the sex ratio was female biased. According to Waage (1986) in general the sex ratio of the gregarious hymenoptera is female biased. The highly female biased sex ratio is advantageous in maintaining mass culture of the parasitoids (Mohandas and Abdurahiman, 1992).

The age specific life table and life table statistics of *N. dipterae* are given in Table 2 and 3. The adult female lived for 15 days and produced maximum number of females on 5th day, but the rate of female progeny production decreased with the age of the female. The calculated finite rate of increase showed that the population of *N. dipterae* increased by 0.50 times ( $\lambda$ ) per female per day. This is in accordance with the life table statistics reported in other uzi fly parasitoids (Nirmala, 1994; Jyothi, 1994).

Table 1: Duration of different stages, fecundity and sex - ratio of *N. dipterae*

Details	Duration
Egg period	1-3 (2.43±0.25)
Larva period	5-6 (5.50±0.89)
Pupal period	7-8 (7.5±0.97)
Adult longevity	21.8±0.98
Average fecundity	105.0±5.29
Sex ratio (F:M)	19:1

\* Figures in parentheses are the mean values

Table 2: Age specific fecundity table for *N. dipterae* (Risbec)

Pivotal age in days (x)	Age specific longevity ( $l_x$ )	No. of females produced per female ( $m_x$ )	$l_x m_x$	$l_x m_x$
1-17	immature stage		—	—
18	1.0	9.26	9.26	166.68
19	1.0	10.24	10.24	194.56
20	1.0	13.08	13.08	261.60
21	0.94	14.86	13.96	293.16
22	0.94	23.50	22.09	485.98
23	0.94	12.00	11.28	259.44
24	0.94	8.50	7.99	191.76
25	0.80	5.36	4.28	107.00
26	0.80	3.44	2.75	71.50
27	0.80	1.70	1.36	36.72
28	0.80	0.94	0.75	21.00
29	0.42	0.50	0.21	6.09
30	0.30	—	—	—
31	0.18	—	—	—
32	0.06	—	—	—
$\sum l_x m_x = 97.25$		$\sum l_x m_x = 2095.49$		

Though this parasitoid parasitising the *Carcelia corvinoids* is recorded (unpublished), the preference of this parasitoid on other beneficial insects are being taken up and if it is found to be useful, this can also be included as one of the component in the integrated approach.

Table 3: Life - table statistics of *N. dipterae* (Risbec)

Parasitoid	$r_m$	$\lambda$	T	$R_0$	DT
<i>Nesolynx dipterae</i>	0.217	0.50	21.04	97.25	3.18



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## Conservation of Spiders in Rice Ecosystem

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### CONSERVATION OF SPIDERS IN RICE ECOSYSTEM

Bio-control agents such as predators parasitoids and insect pathogens form the corner stone of modern integrated pest management (IPM) programme in rice. Spiders are the predators of green leafhopper, brown planthopper and yellow stem borer. Lycosids the dominant group of spiders (Shivamurthappa, 1993) take an average 100 days to reach the adult stage (Gavarra and Raros, 1975; Samal and Misra, 1985) which is almost one cropping season. Hence the conservation of adult spiders after harvest is very important to obtain sufficient inoculum for the next season. The present studies were undertaken to conserve the destructing spider population during and after the harvest of the rice.

Effect of three different modes of crop residue on spider population was studied during summer and kharif seasons of 1995, in Southern Karnataka.

Firstly, different heights of the paddy stubbles were maintained at the time of harvesting viz., 0, 5, 10 and 15 cm from the ground. Increasing the height of the paddy stubbles in the field increased the spider population. Stubbles at the height of 10 and 15 cm harboured significantly higher spiders upto 2.65 and 2.70 per 0.5 m<sup>2</sup> area, respectively as against 0 and 5 cm height stubbles, when recorded a week after harvest.

Table 1: Spider population as influenced by stubbles of different heights

Height of the stubbles (cm)	No. of spiders per 0.5 m <sup>2</sup>
0	1.23 a
5	1.89 b
10	2.65 c
15	2.70 c
SEM	0.058
C. D. (5%)	0.178

\* each value is a mean of 5 observations

\* means followed by the same letter are within the studentized t-range

Table 2: Effect of rice straw bundles on spider population in the mainfield

Sl. No.	No. of bundles per tent	Spiders/0.4 m radius circle	
		A day after harvest	5 day after harvest
1.	No bundles (control)	9.29 a (3.12)	5.71 a (2.47)
2.	2 bundles	18.14 b (4.29)	10.14 b (3.19)
3.	4 bundles	22.43 b (4.75)	13.71 c (3.76)
	SEM	0.202	0.107
	C.D. (5%)	0.621	0.330

\* each value is a mean of 5 observations

\* means followed by the same letter are within the studentized t-range

\* figures in the parenthesis are  $\sqrt{x + 0.5}$  transformed values

Table 3: Population of spiders on rice bunds as influenced by different treatments

Sl.No.	Treatments	No. of spiders per linear m
1.	Clean bund	0.80 a (1.07)
2.	Grassy bund	3.20 b (1.79)
3.	Rice straw on clean bund	4.00 c (2.02)
4.	rice straw on grassy bund	1.00 a (1.30)
	SEM	0.153
	C.D. (5%)	0.445

Another method is by keeping the freshly harvested and threshed rice straw bundles of 15.3 cm diameter in a circle like tents. The treatments were 0, 2 and 4 bundles per  $3 \times 3$  m plots arranged in 0.4 m radius circle. Number of spiders were significantly higher in tents with 4 bundles (13.71 spiders/0.4 m radius circle) followed by the tents with 2 bundles (10.14 spiders/0.4 m radius circle) at five days after harvest. The plots with no straw bundles recorded lowest number of spiders. Number of spiders harboured at five days after harvest were higher when compared to a day after harvest. By considering the number of spiders harboured in 4 bundles tent at one and five days after harvest, it can be concluded that increasing the area for harbour can minimise the cannibalistic and expose to predators which in turn increase the number of spiders conserved (Shephard, 1989).

Lastly, conservation of spiders on the bunds. The different treatments were clean bunds, grassy bunds, covering the clean bunds with crop residue and covering the grassy bunds with crop residue. Covering the clean bunds with crop residue (straw) just prior to harvest conserved highest number of spiders (4/linear m) followed by only grass grown on bunds (3.2/linear m). This is especially useful to conserve spiders from harvest damage and during the application of pesticides.

In all the above cases maximum number of spiders recorded were Lycosids. Having very good reproducing capacity they can contribute for higher number of spiders for next season, especially in areas where the paddy is grown continuously for two seasons. Zheu and Zheng (1984) have also made similar kind of effort to shift the spiders from the leguminous plants in a field borders to the rice field.

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## Effect of Atso Oil Emulsion Sprays in the Control of Mealy Bug *Maconellicoccus hirsutus* Infesting Guava Fruits

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**Abstract:** Atso, an inorganic Oil emulsion spray at 3% provided good control of mealybug, *Maconellicoccus hirsutus* Green. (Pseudococcidae: Homoptera) followed by 2% in 3 days recording an average populations of 0.77 and 3.03 mealybug/cm<sup>2</sup> area of the fruit.

**Keywords:** Atso, *Maconellicoccus hirsutus*, control.

Mealybug cause considerable damage to guava fruits and it is an important pest of guava (Butani, 1979). The normal solution to its management is the use of insecticides, leading to high residues in table fruits that are unacceptable. So, attempts were made using inorganic oil emulsion, Atso to control the mealy bugs. A field trial was conducted with five spray treatments viz., T<sub>1</sub> - 0.5%, T<sub>2</sub> - 1.0%, T<sub>3</sub> - 2.0%, T<sub>4</sub> - 3.0% of Atso oil emulsion and T<sub>5</sub> - untreated check in four replications at college farm. The guava fruit belonging to Hafsi variety were selected for each replications and number of mealybugs both crawless & nymphs/sq.cm was counted before and at 1 & 3 days after spraying mealybugs. Population ranged from 35.79 to 48.40 in untreated control during the trail.

Atso oil emulsion was obtained from inorganic source marketed by Akur Chemicals Pvt. Ltd., Bombay. It is a highly refined oil with a blend of additives and act as a physical poison. There is significant reduction in the mealybug population at 3 days after spraying (Table 1). Atso oil emulsion at 3% recorded 99.29 per cent mortality of mealybugs (0.77 mealybugs/cm<sup>2</sup> area of the fruit) followed by 2% (3.03 mealybug/cm<sup>2</sup>) at 3 days after spraying. Good control of mealybugs might be due to the physical nature of the poison, presence of emulsifiers, might have helped uniform spread of oil there by covering the target area resisting evaporation and ultimately death of the mealybugs due to asphyxiation.

Table 1: Efficacy of Atso oil emulsion sprays in controlling mealybug infesting guava fruits

Concentration	Pre-treatment count	$(x + 0.5)^{-2}/\text{cm}^2$ Mealybug population	
		Days after spraying	
		1	3
0.5%	5.20 <sup>a</sup>	4.02 <sup>d</sup>	2.93 <sup>d</sup>
1.0%	5.95 <sup>a</sup>	3.24 <sup>c</sup>	2.35 <sup>c</sup>
2.0%	5.63 <sup>a</sup>	2.34 <sup>b</sup>	1.86 <sup>b</sup>
3.0%	6.00 <sup>a</sup>	1.79 <sup>a</sup>	1.13 <sup>a</sup>
Control	6.02 <sup>a</sup>	5.76 <sup>c</sup>	5.89 <sup>c</sup>

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

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## Food with Neem Oil Affects Life and Development of Rice Moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae)

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**Abstract:** *Corcyra cephalonica* culture was maintained in neem oil (Azadirachtin, 0.03%) - absorbed crushed jowar grains in four doses (0.25 ml, 0.50 ml, 0.75 ml and 1.00 ml; each dose in 20 gms of food) with initial population of 50 newly hatched larvae per 100 gms of neem-absorbed food in each replication for a dose. Deformed ultimate morphs with prolonged period of development were obtained. The growth inhibition, developmental derangements and mortality increased markedly with the increase of dose.

**Keywords:** Neem oil, feeding, *Corcyra* larvae, development

Entomological importance of neem or margosa plant (*Azadirachta indica* A. Juss.) for its chemical component Azadirachtin is well explored (Coutinho, 1938; Devkumar, *et al.*, 1986). The efficacy of the compound as antifeedant has been mostly assessed on phytophagous insects (Pandey, *et al.*, 1986; Gillott, 1995). Pathak and Krishna (1985) could assess its role in the fertility of *Corcyra cephalonica* by exposing the insect to neem oil vapour. The present study was, therefore, conducted to explore the genesis of antifeedant property of neem by rearing the *Corcyra* larvae upon neem oil-absorbed crushed jowar grains as food.

Larvae of *Corcyra cephalonica* were collected from the stock culture maintained in the laboratory (Deb and Chakravorty, 1981). The antifeedant azadirachtin was treated through the product Nimbecidine containing 0.03% azadirachtin (T. Stanes and Co. Ltd., Coimbatore). The chemical, in required dose, was thoroughly mixed with the food of *Corcyra* larvae. The different doses per 20 gms. of this neem-food were 0.25 ml, 0.50 ml, 0.75 ml and 1.00 ml. Each culture, for a dose, was started with 50 newly hatched first instar larvae per 100 gms. of neem-food in glass jars (13.00 cm × 6.50 cm) covered with pieces of fine nylon nets. The neem-food was replaced once a week. The larvae grew, moulted and emerged as adults by consuming this neem-food. There were three replications for each dose. Control series were maintained



Fig. 1. Typical deformed adults of *C. cephalonica* grown on neem oil mixed food (D – dorsal, V – ventral)

in the same manner with food devoid of Nimbecidine. Metrical data were analysed for testing significance of the individual variations by Duncan's Multiple Range Test (DMRT).

Three categories of ultimate morphs were obtained. These were: apparently normal adults, deformed adults and non-emerged adults. Apparently normal adults were similar, in external morphology, to those of other normal adults emerged from stock culture (Chakravorty, *et al.*, 1989). The deformed and non-emerged adults were characterised by crumpled or twisted wings which were short in length (Fig. 1). The latter morphs were very much ill-grown.

The different developmental stages, *i.e.*, last instar larvae, pupae and adults, from different treatments and control cultures varied significantly with respect to their length, body weight and wing span. The individual comparison between each of the treatments and control showed that only the highest dose, *i.e.*, 1.00 ml/20 gms of food, is the consistently effective one which for all the characters and in all the developmental stages affected growth very significantly ( $P < 0.001$ ). The data for this particular dose and control were: for body length (mm) 10.5 & 12.8, 7.3 & 9.6, 6.3 & 8.6, 7.9 & 9.4; for body weight (mg) 25.4 & 44.2, 23.8 & 35.4, 12.8 & 20.2, 23.2 & 32.8 in ultimate instar larva, pupa, male moth, female moth respectively; for wing span (mm) 15.7 & 23.0, 17.7 & 24.2 in male and female moths respectively. The Nimbecidine (azadirachtin 0.03%) is, thus, an effective growth and development inhibiting chemical when taken with food by newly hatched to mature larvae of *C. cephalonica*.

The experiment further showed that mortality, in all the four food-mixtures, was more than that of control and this increased with the increase of dose. The total duration (larval hatching to adult emergence) was much more in treated individuals. Duration prolonged, even doubled, for morph change. It was apparent that at higher doses development, towards adult formation and emergence, was not favoured. Non-emerged ill-grown adults were only 2.67% in 0.25 ml treatment and with increase of dose such forms were not at all formed. The occurrence of apparently normal adults and deformed adults also sharply declined from 46.66% to 11.33% and 7.33% to 3.33% respectively in doses from 0.25 ml to 1.00 ml (Table 1). The present finding, therefore, suggests that the neem oil, when fed with normal food, serves also as a growth inhibitor in the stored grain pest *Corcyra cephalonica* and, thus, justifies the earlier records of neem seeds as protectant for stored grains (Jotwani and Sircar, 1965).

Table 1: Data on growth, duration of development and emerged morphs due to feeding in different doses of neem-food by the larvae of *C. cephalonia* (Initial population of first instar larva, i.e., n=150)

Treatment (ml. of Nimbecidine/20 gms. of food)	Ultimate resultant morphs (% in parentheses)								Mortality (%)	Duration: Mean±SE in days (Range)
	Apparently normal adult		Deformed adult		Non-emerged adult					
	Male	Female	Male	Female	Male	Female				
0.25	32 (21.33)	38 (25.33)	8 (5.33)	3 (2.00)	4 (2.67)	—	65 (43.33)	44.22±8.81 (35-72)		
0.50	31 (20.67)	16 (10.67)	5 (3.33)	3 (2.00)	—	—	95 (63.33)	48.47±9.25 (38-82)		
0.75	22 (14.67)	10 (6.67)	3 (2.00)	3 (2.00)	—	—	112 (74.67)	54.39±11.52 (45-90)		
1.00	20 (13.33)	12 (8.00)	3 (2.00)	2 (1.33)	—	—	113 (75.33)	55.05±12.65 (38-91)		
Control	48 (32.00)	61 (40.67)	—	—	—	—	41 (27.33)	41.01±5.17 (32-52)		

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